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Dated: October 25, 2005

Signature:

Greta E. Noland
(Greta E. Noland)

Docket No.: 11009/35975C
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Olaf B. Kinstler et al.

Application No.: 09/817,725

Confirmation No.: 1724

Filed: March 26, 2001

Art Unit: 1654

For: N-TERMINALLY CHEMICALLY MODIFIED
PROTEIN COMPOSITIONS AND METHODS

Examiner: B. D. Chism

REQUEST TO CHANGE INVENTORSHIP UNDER 37 C.F.R. §1.48(b)

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby request deletion of two of the individuals originally named as inventors on the above-identified patent application. The names of the two individuals that are to be deleted are Olaf B. Kinstler and Randolph B. De Prince. The names of the two are being deleted because prosecution of the application has resulted in cancellation or amendment of claims so that the two are not inventors of the invention now being claimed.

~~10/28/2005 HLE333 00000028 09817725~~

~~08 FC:1814~~

~~130.00 OP~~

10/28/2005 HLE333 00000028 09817725

09 FC:1464

130.00 OP

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Our check in the amount of the processing fee of \$130.00 set forth in 37 C.F.R. §1.17(i) is submitted herewith. The Commissioner is hereby authorized to charge any fees except the issue fee which may be required, or any credit any overpayment, to Deposit Account Number 13-2855. A duplicate copy of this sheet is enclosed.

Dated: October 25, 2005

Respectfully submitted,

By Greta E. Noland
Greta E. Noland

Registration No.: 35,302
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive, Suite 6300
Sears Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Agent for Applicant

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Dated: 10/25/05 Signature: *Greta E. Noland*
(Greta E. Noland)

Docket No.: 11009/35975C
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Olaf B. Kinstler et al.

Application No.: 09/817,725

Confirmation No.: 1724

Filed: March 26, 2001

Art Unit: 1654

For: N-TERMINALLY CHEMICALLY MODIFIED
PROTEIN COMPOSITIONS AND METHODS

Examiner: B. D. Chism

DECLARATION UNDER 37 C.F.R. §1.131

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I, Nancy Elise Gabriel, declare that:
2. I am named as a co-inventor on United States Patent Application Serial No. 09/817,725. Christine E. Farrar is also a co-inventor on the application.
3. I was employed at Amgen as a research scientist from October, 1988 until November, 2002. One of my responsibilities during that time was supervising a project that resulted in the production of hematopoietic proteins modified at the N-terminus with a polyethylene glycol moiety. I have been informed that such proteins are presently being claimed in the application.
4. Christine E. Farrar conducted laboratory work associated with the project and that work is described in her Amgen Notebook Nos. 5575, 5576 and 6951 attached hereto as Exhibits A, B and C, respectively. Dates have been redacted from the exhibits. Her notebook pages, specifically referenced below and dated prior to October 1993, demonstrate that we had produced and recognized the value of hematopoietic proteins modified at the N-terminus with a polyethylene glycol moiety.
5. Granulocyte colony-stimulating factor (G-CSF) was one of the hematopoietic proteins we were modifying with polyethylene glycol (PEG) prior to October 1993.

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6. Recombinant human met-G-CSF (referred to as "rhG-CSF" or "rh-G-CSF" herein) was prepared according to methods in the Souza patent, U.S. Patent No. 4,810,643. The rhG-CSF had the amino acid sequence (encoded by the DNA sequence) shown below.

```

ATG ACT CCA TTA GGT CCT GCT TCT TCT CTG CCG CAA AGC TTT CTG
M   T   P   L   G   P   A   S   S   L   P   Q   S   F   L

CTG AAA TGT CTG GAA CAG GTT CGT AAA ATC CAG GGT GAC GGT GCT
L   [K] C   L   E   Q   V   R   [K] I   Q   G   D   G   A

GCA CTG CAA GAA AAA CTG TGC GCT ACT TAC AAA CTG TGC CAT CCG
A   L   Q   E   [K] L   C   A   T   Y   [K] L   C   H   P

GAA GAG CTG GTA CTG CTG GGT CAT TCT CTT GGG ATC CCG TGG GCT
E   E   L   V   L   L   G   H   S   L   G   I   P   W   A

CCG CTG TCT TCT TGT CCA TCT CAA GCT CTT CAG CTG GCT GGT TGT
P   L   S   S   C   P   S   Q   A   L   L   Q   A   G   C

CTG TCT CAA CTG CAT TCT GGT CTG TTC CTG TAT CAG GGT CTT CTG
L   S   Q   L   H   S   G   L   F   L   Y   Q   G   L   L

CAA GCT CTG GAA GGT ATC TCT CCG GAA CTG GGT CCG ACT CTG GAC
Q   A   L   E   G   I   S   P   E   L   G   P   T   L   D

ACT CTG CAG CTA GAT GTA GCT GAC TTT GCT ACT ACT ATT TGG CAA
T   L   Q   L   D   V   A   D   F   A   T   T   I   W   Q

CAG ATG GAA GAG CTC GGT ATG GCA CCA GCT CTG CAA CCG ACT CAA
Q   M   E   E   L   G   M   A   P   A   L   Q   P   T   Q

GGT GCT ATG CCG GCA TTC GCT TCT GCA TTC CAG CGT CGT GCA GGA
G   A   M   P   A   F   A   S   A   F   Q   R   R   A   G

GGT GTA CTG GTT GCT TCT CAT CTG CAA TCT TTC CTG GAA GTA TCT
G   V   L   V   A   S   H   L   Q   S   F   L   E   V   S

TAC CGT GTT CTG CGT CAT CTG GCT CAG CCG TAA TAG
Y   R   V   L   R   H   L   A   Q   P   *   *

```

The rhG-CSF had one alpha amino reactive group (amino acid residue 1) and four epsilon amino reactive groups (amino acid residues boxed above) available for modification with a PEG moiety.

7. A 10 mg/ml solution of the above rh-G-CSF, in 100 mM Bicine pH 8.0, was added to solid SCM-MPEG (N-hydroxy succinimidyl esters of carboxymethyl methoxy polyethylene glycol) (Union Carbide) with an average molecular weight of 6000 Daltons to

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give a 1.5 molar excess of SCM-MPEG to rh-G-CSF. After one hour with gentle stirring, the mixture was diluted to 2 mg/ml with sterile water, and the pH was adjusted to 4.0 with dilute HCl. The reaction was carried out at room temperature. At this stage, the reaction mixture consisted mainly of three forms of mono-pegylated rh-G-CSF, some di-pegylated rh-G-CSF, unmodified rh-G-CSF and reaction bi-product (N-hydroxy succinimide). See pages 82 and 83 of Notebook No. 5576.

8. The three forms of monopegylated rh-G-CSF were separated from each other using ion exchange chromatography. The reaction mixture was loaded (1 mg protein/ml resin) onto a Pharmacia S Sepharose FF column (Pharmacia XK50/30 reservoir, bed volume of 440 ml) equilibrated in buffer A (20 mM sodium acetate, pH 4.0). The column was washed with 3 column volumes of buffer A. The protein was eluted using a linear gradient from 0-23% buffer B (20 mM sodium acetate, pH 4.0, 1M NaCl) in 15 column volumes. The column was then washed with one column volume of 100% buffer B and reequilibrated with 3 column volumes of buffer A. The flow rate for the entire run was maintained at 8 ml/min. The eluent was monitored at 280 nm and fractions were collected. Fractions containing the individual monopegylated species were pooled according to Figure 1A of the patent application. See page 86 of Notebook No. 5576. These pools were concentrated with a 350 mL Amicon stirred cell using a YM10 76 mm membrane. See pages 83-89 of Notebook No. 5576.

9. Pooled fractions from the ion exchange chromatography were subjected to size exclusion chromatography to separate di-pegylated species from monopegylated species. Typically, 5-10 mg in 2-5 ml of solution were loaded onto a 120 ml Pharmacia Superdex 75 HR 16/60 column equilibrated with 20 mM sodium acetate pH 4.0. The column was run at 1.5 ml/min for 100 min. Two ml fractions were collected. The protein content of the eluent was monitored at 280 nm. Fractions from separated peaks were pooled. See pages 1-13 of Notebook No. 6951. Page 5 of the notebook reports enough material had been obtained so that the characteristics of the three forms of monopegylated rh-G-CSF could be analyzed.

10. As described in Example 1 of the patent application, five analyses were done to characterize the three forms: (1) SDS-Page, (2) Size exclusion chromatography HPLC ("SEC HPLC"), (3) peptide mapping analysis, (4) *in vitro* G-CSF bioassay, and (5) *in vivo* testing in hamster.

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11. Peptide mapping and N-terminal sequence analyses (described at Application page 33, line 21 through page 36, line 3) revealed that one form, "Mono-PEG-1", was an N-terminally monopegylated conjugate. See pages 20 and 21 of Notebook No. 6951.

12. The N-terminally monopegylated rhG-CSF and the other two monopegylated samples were tested for activity in an *in vitro* mitogenic assay utilizing a G-CSF dependent clone of murine 32D cells. Cells were maintained in Iscoves medium containing 5% FBS and 20 ng/ml rhG-CSF. Prior to sample addition, cells were prepared by rinsing twice with growth medium lacking rhG-CSF. An extended twelve point rhG-CSF standard curve was prepared, ranging from 48 to 0.5 ng/ml (equivalent to 4800 to 50 IU/ml). Four dilutions, estimated to fall within the linear portion of the standard curve, (1000 to 3000 IU/ml), were prepared for each sample and run in triplicate. Because of their apparent lower activity *in vitro*, the pegylated rhG-CSF samples were diluted approximately 4-10 times less. A volume of 40 µl of each dilution of sample or standard was added to appropriate wells of 96 well microtiter plate containing 10,000 cells/well. After forty-eight hours at 37°C and 5.5% CO₂, 0.5 µmCi of methyl-³H-thymidine was added to each well. Eighteen hours later, the plates were then harvested and counted. A dose response curve (log rhG-CSF concentration vs. CPM-background) was generated and linear regression analysis of points which fall in the linear portion of the standard curve was performed. Concentrations of unknown test samples were determined using the resulting linear equation and correction for the dilution factor. As can be seen from Figure 4 of the patent application, of the three monopegylated species, N-terminally monopegylated G-CSF demonstrated the highest *in vitro* biological activity. The N-terminally monopegylated material had 68% of the activity of non-modified rhG-CSF. See page 17 of Notebook No. 6951.

13. *In vivo* testing confirmed the activity of the N-terminally monopegylated material. The *in vivo* testing was carried out by dosing male golden hamsters with a 0.1 mg/kg of sample, using a single subcutaneous injection. Four animals were subjected to terminal bleeds per group per time point. Serum samples were subject to a complete blood count on the same day that the samples were collected. The average white blood cell counts were calculated. As can be seen in Figures 5A and 5B of the patent application, the response from each material peaks after one day following a single subcutaneous injection of 0.1 mg/kg. Two of the monopegylated materials, (N-terminal and Lys-35) showed prolonged responses, while the response for the protein pegylated at lysine-41 showed no increase in *in*

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vivo activity over unmodified rhG-CSF (indeed it shows less, Application Figure 5B). See pages 18 and 19 and pages 74-77 of Notebook No. 6951.

14. We thus had made a hematopoietic protein modified at the N-terminus with a PEG moiety which had a prolonged *in vivo* biological activity as evidenced by the aforementioned notebook pages which are dated prior to October 1993.

15. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

October 17, 2005
Date

Nancy Elise Gabriel
Nancy Elise Gabriel

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6413

LABORATORY NOTEBOOK

№ 5575

5575

AMGEN

REDACTED

Box 0608

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REDACTED

NOTEBOOK NO. 5575
ISSUED TO Christine Farrar
ON _____ 19____
DEPARTMENT 215
RETURNED _____ 19____

"MICROFILMED"

DATE _____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

INSTRUCTIONS

1. The primary purpose of this notebook is to protect your and the Company's Patent-Rights by keeping records of all original work in a form acceptable as evidence if any legal conflict arises.
2. When starting a page, enter the title, project number, and book number. Use ink for permanence—no pencil. Record your work as you progress, including any spur-of-the-moment ideas which may be developed later. Do not make notes on loose paper to be recopied. Use the blank lefthand page for calculations so they will be available if you want to re-check them. Record your work in such a manner that a co-worker can continue from where you stop. You might be ill and to protect your priority it could be urgent that the work continue while you are absent.
3. Give a complete account of your experiments and the results, both positive and negative, including your observations. Record all diagrams, layouts, plans, procedures, new ideas, or anything pertinent to your work including the details of any discussions with suppliers, or other people outside the Company. Do not try to erase any incorrect entries; draw lines deleting them, note the corrections, sign and date the changes. This extra care is worthwhile because of the necessity of original data to prove priority of new discoveries.
4. After entering your data, sign and date the entries. Explain your work to at least two witnesses who are not co-inventors, and have them sign and date the pages in the place provided. Record the names of operators and witnesses present during any demonstration and have at least two witnesses sign the page. If no witnesses are present during an experiment of importance, repeat it in the presence of two witnesses.
5. This notebook and its contents are the exclusive property of the Company. It is confidential and the contents are not to be disclosed to anyone unless authorized by the Company. You must return it when completed, upon request, or upon termination of employment. It should be kept in a protected place. If loss occurs notify your supervisor immediately, and make a written report describing the circumstances of the loss.

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Project No. 11415Book No. 5575TITLE High MW PEG-GCSF SDS PAGEFrom Page No. X

REDACTED

Date: _____

Operator: Chris

G-CSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

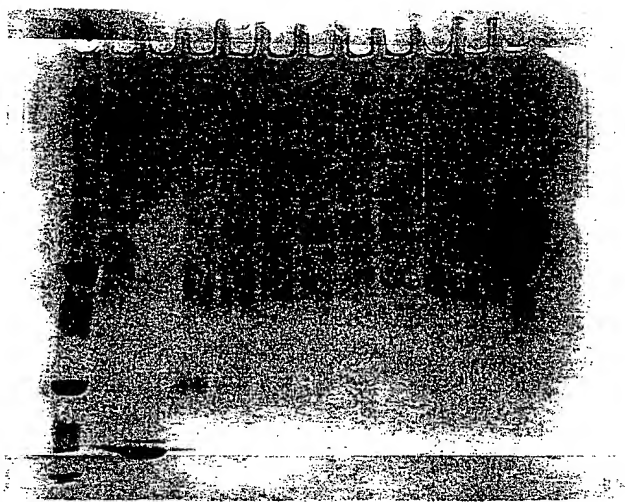
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW std low	1			5.0	
MW std hi	2			5.0	
GCSF	3	2.70	2.025	3.0	6.08
43C 6x	4	1.00	0.750	8.0	6.00
43C 8x	5	1.00	0.750	8.0	6.00
43C 10x	6	1.00	0.750	8.0	6.00
43C 12x	7	1.00	0.750	8.0	6.00
43C 14x	8	1.00	0.750	8.0	6.00
43C 16x	9	1.00	0.750	8.0	6.00
43C 18x	10	1.00	0.750	8.0	6.00
PG12301	11	1.00	0.750	8.0	6.00
EN 080	12	5.20	3.900	1.5	5.85

To Page No. X

Witnessed & Understood by me,

William Callahan

Date _____

Invented by

Christine Furrer

Recorded by _____

Date _____

111

TITLE PEG-BDNF SDS PAGEProject No. 12101Book No. 5575From Page No. X

REDACTED

Date:

Operator: Chris

BDNF gel 1

NB No.:

4-20% Gradient Mini Gel

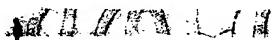
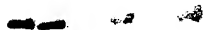
Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: PDGF procedure

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
Pharm STDs 1:1 with dye	1			2.0	
BDNF std 12-1-91	2	2.14	1.605	3.7	5.94
control BDNF	3	1.00	0.750	8.0	6.00
	4				
Peg BDNF	5	0.88	0.660	9.0	5.94
	6				
Peg BDNF	7	0.88	0.660	9.0	5.94
	8				
	9				
	10				
	11				
	12				



To Page No. X

Witnessed & Understood by me,

William Collopy

Date

Invented by

Christine Farrow

Date

Recorded by

TITLE High MW PEG-GCSF SDS PAGE

Project No. 11415

Book No. 5575

3

From Page No. X

REDACTED

Date:

Operator: Chris

G-CSF gel 2

NB No.:

4-20% Gradient Mini Gel

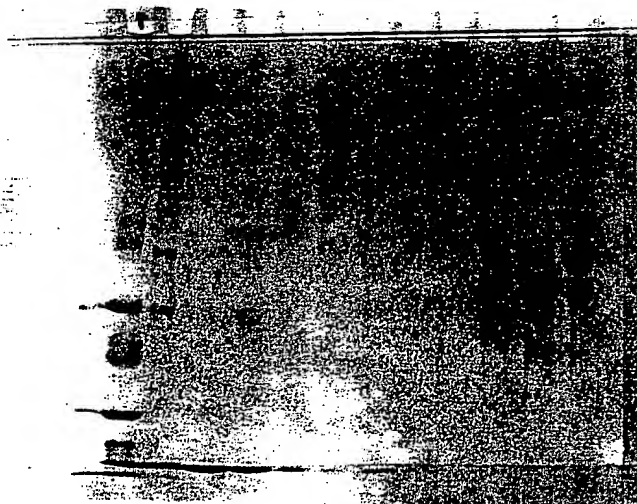
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW std low	1			5.0	
MW std hi	2			5.0	
GCSF	3	2.70	2.025	3.0	6.08
ddi 6x-1	4	1.00	0.750	8.0	6.00
ddi 6x-5	5	1.00	0.750	8.0	6.00
ddi 6x-10	6	1.00	0.750	8.0	6.00
ddi 12x-1	7	1.00	0.750	8.0	6.00
ddi 12x-5	8	1.00	0.750	8.0	6.00
ddi 12x-10	9	1.00	0.750	8.0	6.00
PG12301	10	1.00	0.750	8.0	6.00
EN079	11	5.00	3.750	1.6	6.00
EN 080	12	5.20	3.900	1.5	5.85



To Page No. X

Witnessed & Understood by me,

William Allison

Date

Invented by

Christina Fomara

Date

Recorded by

Project No. 11802

Book No. 5575

TITLE PEG-MI IEF

4

From Page No. X

Ampholine[®] PAGplate

Experimental result form

LKB

pH range 3-9

Anode Electrode Solution 1M H₂SO₄

Cathode Electrode Solution 1M NaOH

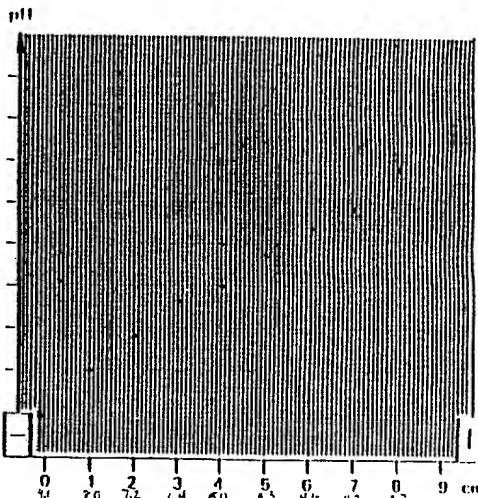
Formamide staining
SDS A0330 with 250R

Date

Experiment No. PEG-MI

Operator CF

Sample No.	Sample description	Conc. (mg/ml)	Volume (μl)	Position						
				1	2	3	4	5	6	7
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	M1 .3	.5	40							
12	M1 .6	.5	40							
13	M1 .9	.5	40							
14	M1 1.2	.5	40							
15	M1 1.5	.5	40							
16	M1 3.0	.5	40							
17	Native ChoMI	.5	40							
18	PEG-MI 4713-39	.46	26							
19										
20										
21										
22										
23										
24										



Electrofocusing data

Cooling temperature

-10 °C

pH measured at

10 °C

Time	Voltage	Current	Power
START	500V	42 mA	5W
20 min	800V	6 mA	5W
Start Samples	750V	7 mA	5W
30 min	1500V	3.5 mA	5W
60 min	1700V	2.6 mA	5W
END			

95 56 (M1)

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Ferrar

Date

Recorded by

111

TITLE AM-MI SDS PAGEProject No. 11802Book No. 5575

5

From Page No. X

REDACTED

Date: _____

Operator: Chris

MI gel 1

NB No.: _____

4-20% Gradient Mini Gel

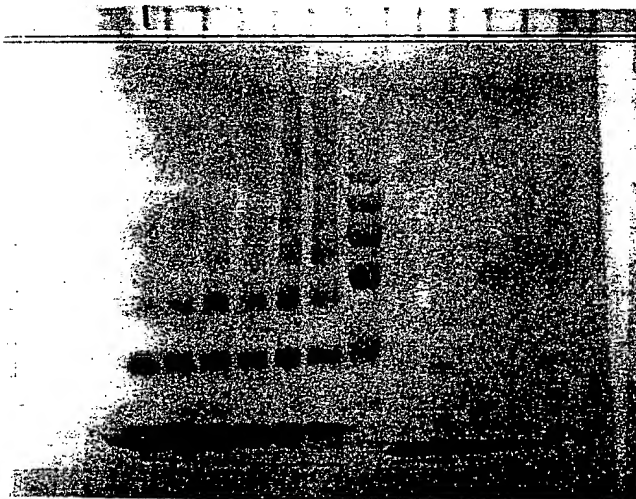
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
AM-MI .3	1	0.50	0.375	15.0	5.63
AM-MI .6	2	0.50	0.375	15.0	5.63
AM-MI .9	3	0.50	0.375	15.0	5.63
AM-MI 1.2	4	0.50	0.375	15.0	5.63
AM-MI 1.5	5	0.50	0.375	15.0	5.63
AM-MI 3.0	6	0.50	0.375	15.0	5.63
PEG-MI lot 4713-39	7	0.76	0.570	10.0	5.70
MI lot R6	8	1.25	0.938	6.0	5.63
MI lot R6	9	.50	.375	15.0	5.63

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrar

Date

Recorded by

Project No. 11415
 Book No. 5575

TITLE PEG - GCSF SDS PAGE

From Page No. _____

REDACTED

Date: _____

Operator: Chris

G-CSF gel2

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

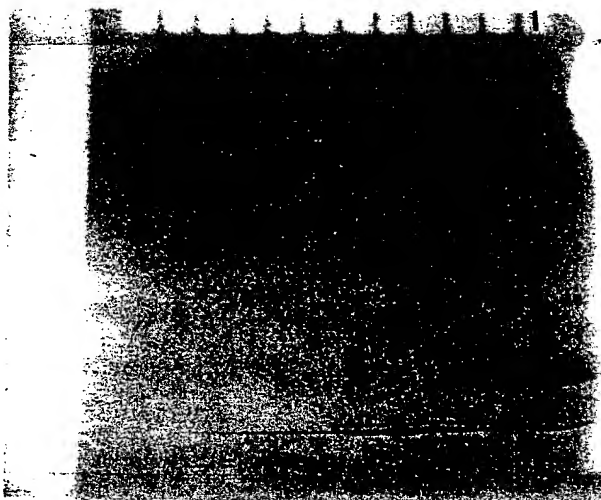
constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
mono PEG 6000	1	0.55	0.413	15.0	6.19
di PEG 6000	2	0.35	0.263	15.0	3.94
PEG-rhu-GCSF lot 4656-47	3	1.00	0.750	8.0	6.00
PEG-rhu-GCSF lot 4656-47	4	1.00	0.750	8.0	6.00
G-CSF STD lot 636JOA	5	0.30	0.225	20.0	4.50
G-CSF STD lot 636JOA	6	0.30	0.225	10.0	2.25

di PEG 6000 concentrated from .14^{mg}/ml \rightarrow .35^{mg}/ml using Millipore UltraFree-MC 10,000 NMWL Filter Unit
 $(.14 \text{ mg/ml})(.100 \text{ ml}) = (x \text{ mg/ml})(.040 \text{ ml})$
 $x = .35 \text{ mg/ml}$



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnan

Date

Recorded by

From Page No. X

REDACTED

Ampholine[®] PAGplate

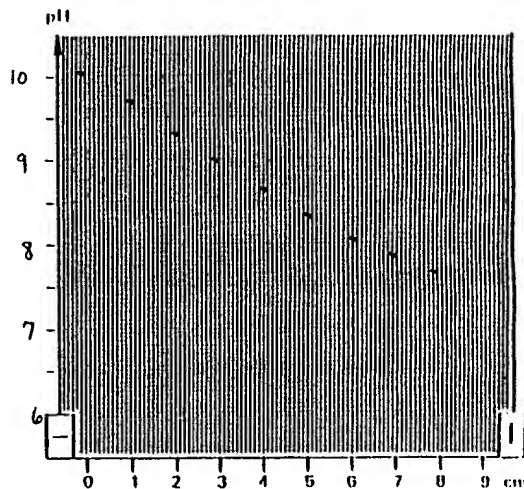
Experimental result form

LKB

pH range R-11 (1 part 7.9; 4 part 9.4 Ampholine)
Anode Electrode Solution 27a 6.9 Ampholyte
Cathode Electrode Solution 1M NaOH

Date
Experiment No. AM-MI
Operator DE

Sample No.	Sample description	Conc. (mg/ml)	Volume (μl)	Position						
				1	2	3	4	5	6	7
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	MI 1.6	.5	40	X	X					
12	MI 1.9	.5	35	X	X					
13	MI 1.2	.5	40	X	X					
14	MI 1.5	.5	40	X	X					
15	MI 3	.5	40	X	X					
16	IEF 10.5-5 (Pharmacia)		10	X						
17	Cytochrome C, Trypsinogen	2	5ea	X						
18										
19										
20										
21										
22										
23										
24										



Electrofocusing data

Cooling temperature 10 °C

pH measured at 10 °C

Time	Voltage	Current	Power
START: 40	500V	18mA	8W
11:33	1000V	7mA	8W
2:08	1000V	8mA	8W
2:58	1400V	5mA	8W
3:08	1600V	5mA	8W
3:38	1780V	5mA	8W
END: 4:00	1800V	4mA	8W

95 58 0913



To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrar

Recorded by

Date

Project No. 11410Book No. 5575TITLE Mono & Di Pegylated GCSF SDS PAGEFrom Page No. 7

REDACTED

GCSF-3

Date: _____

Operator: Chris

G-CSF gel1

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
6X RXN MIX	1	2.00	1.500	4.0	6.00
MONO 1.5X	2	0.55	0.413	14.5	6.00
(54-59)2-303-05 fxn24	3	1.81	1.358	4.4	6.00
fxn25	4	2.46	1.860	3.2	6.00
fxn26	5	1.96	1.470	4.1	6.00
fxn27	6	0.91	0.683	8.8	6.00
(36-37)2-303-03 fxn19	7	0.64	0.480	12.5	6.00
fxn20	8	1.14	0.855	7.0	6.00
fxn21	9	0.92	0.690	8.7	6.00
(DI REC)2-303-03 fxn19	10	0.15	0.113	17.8	2.00
fxn20	11 12	0.22	0.165	18.2	3.00
fxn21	12 11	0.15	0.113	17.8	2.00

To Page No. 8

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrar

Date

Recorded by

T T T

TITLE TM Regulated GCSF SDS PAGEProject No. 11415Book No. 5575

9

From Page No. X

REDACTED

GCSF-4

Date: _____

Operator: Chris

G-CSF gel2

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code		Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
6X RXN MIX		1	2.06	1.500	4.0	6.00
(3- 7)2-303-02	fxn10	2	0.08	0.060	16.7	1.00
	fxn11	3	0.32	0.240	16.7	4.00
	fxn12	4	0.14	0.105	19.0	2.00
(11-12)2-303-02	fxn17	5	0.42	0.315	15.9	5.00
	fxn18	6	1.02	0.765	7.8	6.00
	fxn19	7	1.01	0.758	7.9	6.00
	fxn20	8	0.52	0.390	15.4	6.00
GCSF lot 636JOA		9	0.30	0.225	17.8	4.00
HW std low		10			2.0	
HW std hi		11			5.0	

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnon

Date

Recorded by

Project No. 11415Book No. 5575TITLE Various Pegylated GCSF SDS PAGE -1From Page No. 1

REDACTED

PEG-GCSF-1

Date: _____

Operator: Chris

PEG-GCSF gel1

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1	1.00	0.750	6.7	5.00
LOW MW STD	2	1.00	0.750	6.7	5.00
4656-35 PO423091	4	1.00	0.750	6.7	5.00
4656-47 15X w/ 43C	5	1.00	0.750	6.7	5.00
ENZON-79	7	1.00	0.750	6.7	5.00
ENZON-83	8	1.00	0.750	6.7	5.00
5740-5 MONO-6PEG-GCSF	10	0.55	0.413	12.1	5.00
5740-6 DI-6PEG-GCSF	11	0.26	0.194	20.7	4.00
5740-7 TRI-6PEG-GCSF	12	0.32	0.240	20.8	5.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnan

Date

Recorded by

FTI

TITLE Various Pegylated GCSF SDS PAGE - 2Project No. 11415
Book No. 5575

11

From Page No. X

REDACTED

PEG-GCSF-2

Date: _____

Operator: Chris

PEG-GCSF gel2

NB No.: _____

4-20% Gradient Mini Gel

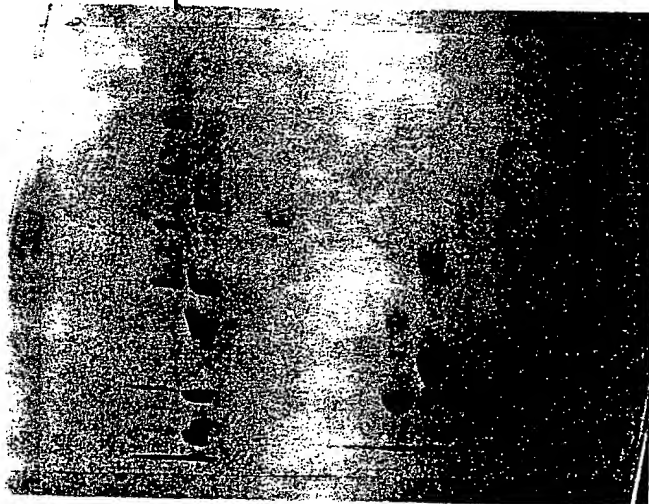
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1	1.00	0.750	6.7	5.00
LOW MW STD	2	1.00	0.750	6.7	5.00
4656-71 MONO-50PEG-G	4	0.44	0.327	15.3	5.00
4656-72 DI-50PEG-G	5	0.55	0.409	12.2	5.00
4626-24 5K LOW	7	0.40	0.296	16.9	5.00
4656-26 10K LOW	8	0.34	0.256	19.6	5.00
5070-26 5K HI	10	0.30	0.221	20.3	4.50
5070-32 10K HI	11	0.35	0.260	19.3	5.00

To Page No. X

Witnessed & Understood by me,

William Collopy

Date

Invented by

Christine Farnon

Date

Recorded by

Project No. 11415Book No. 5575TITLE pH/ratio Survey of SCM-MPEG SOS PAGEFrom Page No. X

REDACTED

SCM-MPEG

Date: _____

Operator: Chris

SCM-MPEG gel1

NB No.: _____

4-20% Gradient Mini Gel

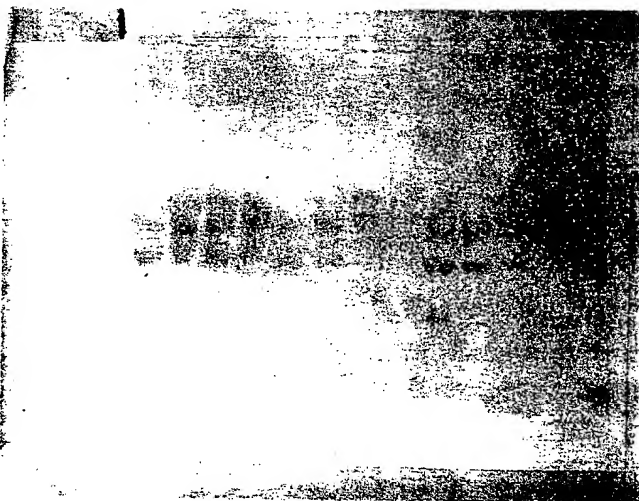
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
pH 8.0 12X	1	1.00	0.750	8.0	6.00
pH 8.0 18X	2	1.00	0.750	8.0	6.00
pH 8.0 24X	3	1.00	0.750	8.0	6.00
pH 8.0 36X	4	0.75	0.563	10.7	6.00
pH 9.0 12X	5	1.00	0.750	8.0	6.00
pH 9.0 18X	6	1.00	0.750	8.0	6.00
pH 9.0 24X	7	1.00	0.750	8.0	6.00
pH 9.0 36X	8	1.00	0.750	8.0	6.00
pH 10.0 12X	9	1.00	0.750	8.0	6.00
pH 10.0 18X	10	1.00	0.750	8.0	6.00
pH 10.0 24X	11	1.00	0.750	8.0	6.00
pH 10.0 36X	12	1.00	0.750	8.0	6.00



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Jansen

Recorded by

Date

From Page No. X

SCM-MPEG CONTROLS

REDACTED

Date: _____

Operator: Chris

SCM-MPEG gel2

NB No.: _____

4-20% Gradient Mini Gel

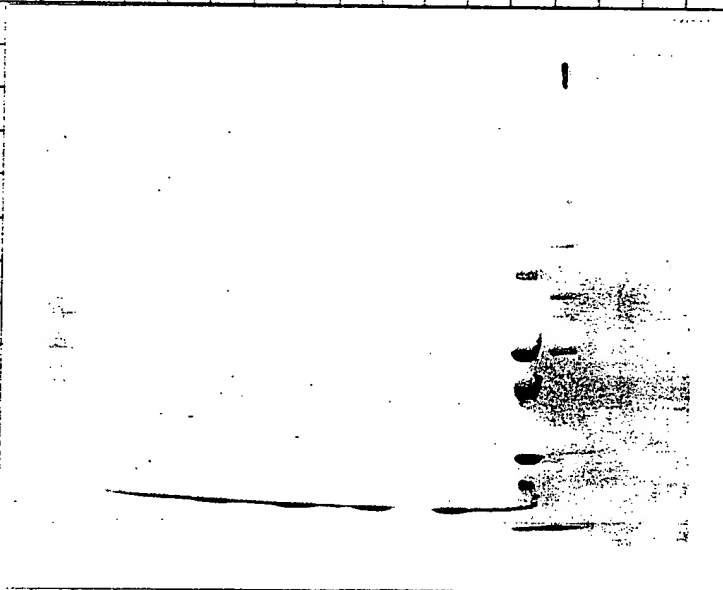
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1	1.00	0.750	5.0	3.75
LOW MW STD	2	1.00	0.750	5.0	3.75
GCSF IN WFI pH 4.0	4	1.00	0.750	8.0	6.00
GCSF pH 8.0	6	1.00	0.750	8.0	6.00
GCSF pH 9.0	8	1.00	0.750	8.0	6.00
GCSF pH 10.0	10	1.00	0.750	8.0	6.00



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnan

Date

Recorded by

Project No. 11415Book No. 5575TITLE pH 8 SCM-MPEG SDS PAGE -1From Page No. 4

REDACTED

SCM-MPEG pH8-1

Date: _____

Operator: Chris

SCM-MPEG pH 8 gel1

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
GCSF	1	1.00	0.750	4.0	3.00
12X 9	2	0.50	0.375	8.0	3.00
12X 10	3	0.25	0.188	16.0	3.00
12X 11-12	4	0.35	0.264	11.4	3.00
12X 13	5	0.10	0.075	22.0	1.65
12X 14-15	6	0.10	0.075	22.0	1.65
4656-47	7	1.00	0.750	4.0	3.00
18X 8-9	8	0.70	0.525	5.7	3.00
18X 10	9	0.12	0.090	22.0	1.98
18X 11-12	10	0.15	0.113	22.0	2.48
LOW MW STD	11			5.0	

To Page No. 1

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnham

Date

Recorded by

From Page No. 1

REDACTED

SCM-MPEG pH8-2

Date: _____

Operator: Chris

SCM-MPEG pH 8 gel 2

NB No.: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Silver Stain SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
GCSF	1	1.00	0.750	4.0	3.00
12X 9	2	0.50	0.375	8.0	3.00
12X 10	3	0.25	0.188	16.0	3.00
12X 11-12	4	0.35	0.264	11.4	3.00
12X 13	5	0.10	0.075	22.0	1.65
12X 14-15	6	0.10	0.075	22.0	1.65
4656-47	7	1.00	0.750	4.0	3.00
18X 8-9	8	0.70	0.525	5.7	3.00
18X 10	9	0.12	0.090	22.0	1.98
18X 11-12	10	0.15	0.113	22.0	2.48
LOW MW STD	11			2.0	

To Page No. x

Witnessed & Understood by me,

William Callahan

Date

Invented by

William Callahan

Date

Recorded by

Project No. 11415Book No. 5575TITLE pH 8 SCM-MPEG SDS PAGE-3From Page No. A

REDACTED

SCM-MPEG pH8-3

Date: _____

Operator: Chris

SCM-MPEG pH 8 gel 3

NB No.: _____

4-20% Gradient Mini Gel

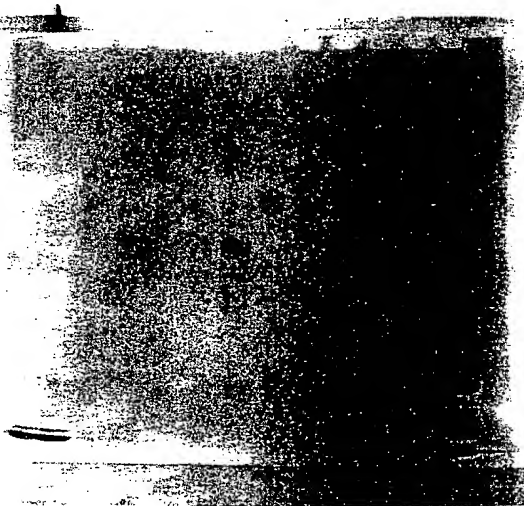
Running Conditions

constant current: 25 mA

all non-reduced

Coomasie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
GCSF	1	1.00	0.750	4.0	3.00
24X 8-9	2	0.35	0.263	11.4	3.00
24X 10-11	3	0.50	0.375	8.0	3.00
24X 12-13	4	0.10	0.075	22.0	1.65
24X 14-15	5	0.10	0.075	22.0	1.65
4656-47	6	1.00	0.750	4.0	3.00
36X 9	7	0.60	0.450	6.7	3.00
36X 10	8	0.20	0.150	20.0	3.00
36X 11-12	9	0.35	0.263	11.4	3.00
36X 13	10	0.10	0.075	22.0	1.65
36X 14-15	11	0.10	0.075	22.0	1.65
LOW MW STD	12			5.0	



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

William Callahan

Date

Recorded by

TITLE pH 8 SCM-MPEG SDS PAGE - 4

Project No. 11415
Book No. 5575

17

From Page No. X

REDACTED

SCM-MPEG pH8-4

Date: _____

Operator: Chris
SCM-MPEG pH 8 gel 4

NB No.: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Silver Stain: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
GCSF	1	1.00	0.750	4.0	3.00
24X 8-9	2	0.35	0.263	11.4	3.00
24X 10-11	3	0.50	0.375	8.0	3.00
24X 12-13	4	0.10	0.075	22.0	1.65
24X 14-15	5	0.10	0.075	22.0	1.65
4656-47	6	1.00	0.750	4.0	3.00
36X 9	7	0.60	0.450	6.7	3.00
36X 10	8	0.20	0.150	20.0	3.00
36X 11-12	9	0.35	0.263	11.4	3.00
36X 13	10	0.10	0.075	22.0	1.65
36X 14-15	11	0.10	0.075	22.0	1.65
LOW MW STD	12			2.0	



To Page No. _____

Witnessed & Understood by me,

William Allen

Date

Invented by

Christine Jones

Date

Recorded by

Project No. 11807Book No. 6575TITLE AM-MI MONO & XLINKED SDS PAGEFrom Page No. 2

REDACTED

MI-2

Date: _____

Operator: Chris

MI gel 1

NB No.: _____

4-20% Gradient Mini Gel

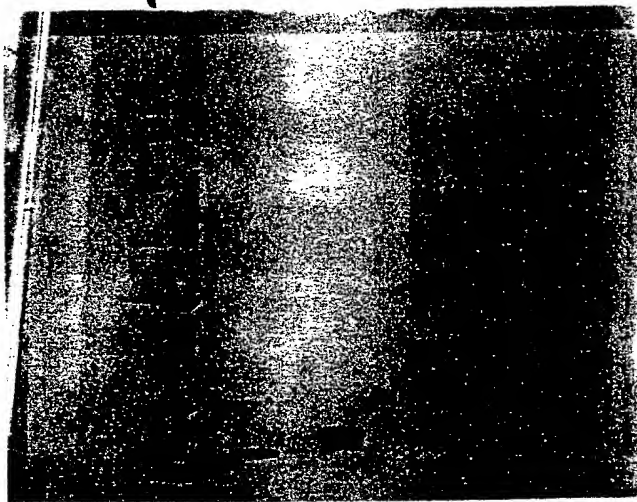
Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
MI lot R7	4	1.00	0.75	5.3	4.00
AM-MI-MONO 4713-59A	6	1.00	0.75	5.3	4.00
AM-MI-XLINKED 4713-59B	8	0.75	0.56	7.1	4.00
PEG-MI 4713-65	10	1.00	0.75	8.0	6.00

To Page No. 2

Witnessed & Understood by me,

William Callahan

Date

Invented by

Matthew J. Farni

Date

Recorded by

From Page No. X

REDACTED

Ampholine[®] PAGplate

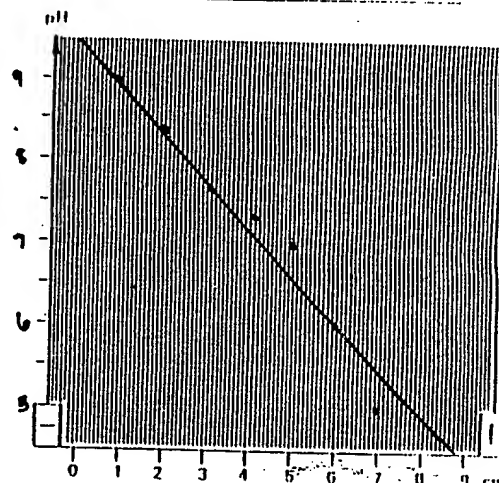
Experimental result form

LKB

pH range 3.5-9
Anode Electrode Solution 1M H₂SO₄
Cathode Electrode Solution 1M NaOH

Date _____
Experiment No. AM-MI
Operator CE

Sample No.	Sample description	Conc. (mg/ml)	Volume (μl)	Position						
				1	2	3	4	5	6	7
1										
2										
3										
4										
5										
6										
7										
8	MI lot R7	1	10	X						
9	MI lot R7	1	10						X	
10	AM-MI-MONO 4713-59A	1	10	X						
11	AM-MI-MONO 4713-59A	1	10						X	
12	AM-MI-XLINKED 4713-59B	.75	13.3	X						
13	AM-MI-XLINKED 4713-59B	.75	13.3						X	
14	PEG-MI 4713-65	1	10	X						
15	PEG-MI 4713-65	1	10						X	
16	Cytochrome C	1	10		X					
17										
18										
19										
20										
21										
22										
23										
24										



Electrofocusing data

Cooling temperature

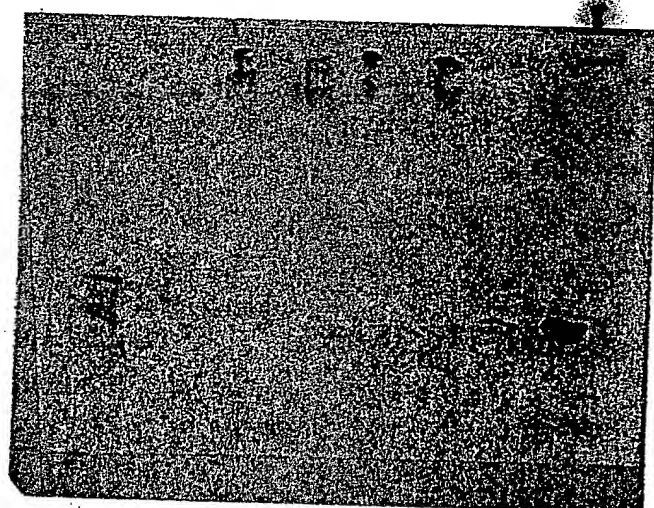
~10 °C

pH measured at

~10 °C

Time	Voltage	Current	Power
START: 20	400V	14mA	6W
2:00	1200V	10mA	6W
2:30	1600V	7mA	6W
3:00	1800V	6mA	6W
3:30	2000V	4mA	1W
END			

95 56 0111



To Page No. X

Witnessed & Understood by me,

William Collamore

Date

Invented by

William Collamore

Recorded by

Date

From Page No. X

REDACTED

BDNF-1

Date: _____

Operator: Chris

BDNF-1

NB No.: _____

4-20% Gradient Mini Gel

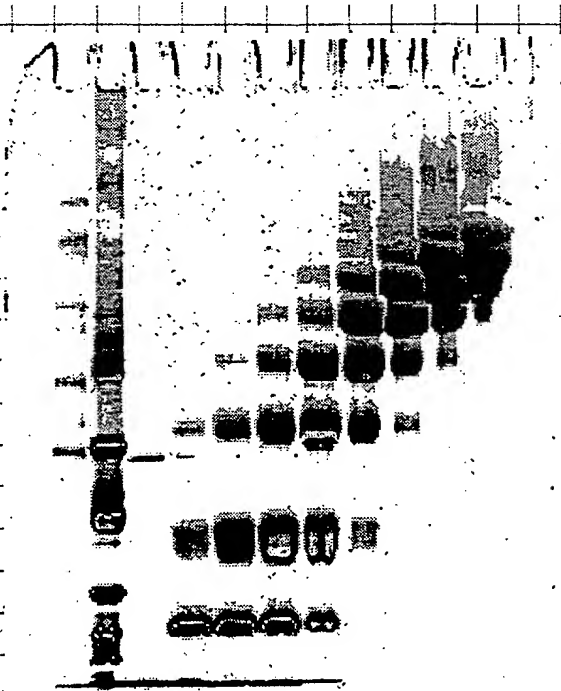
Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
BDNF	3	1.90	1.425	2.1	3.00
BDNF 1X	4	2.00	1.50	4.0	6.00
BDNF 2X	5	2.00	1.50	4.0	6.00
BDNF 3X	6	2.00	1.50	4.0	6.00
BDNF 4X	7	2.00	1.50	4.0	6.00
BDNF 6X	8	2.00	1.50	4.0	6.00
BDNF 8X	9	2.00	1.50	4.0	6.00
BDNF 10X	10	2.00	1.50	4.0	6.00
BDNF 12X	11	2.00	1.50	4.0	6.00



To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Recorded by

Date

From Page No. X

REDACTED

NT3-1

Date: _____

Operator: Chris

NT3-2

NB No.: _____

4-20% Gradient Mini Gel

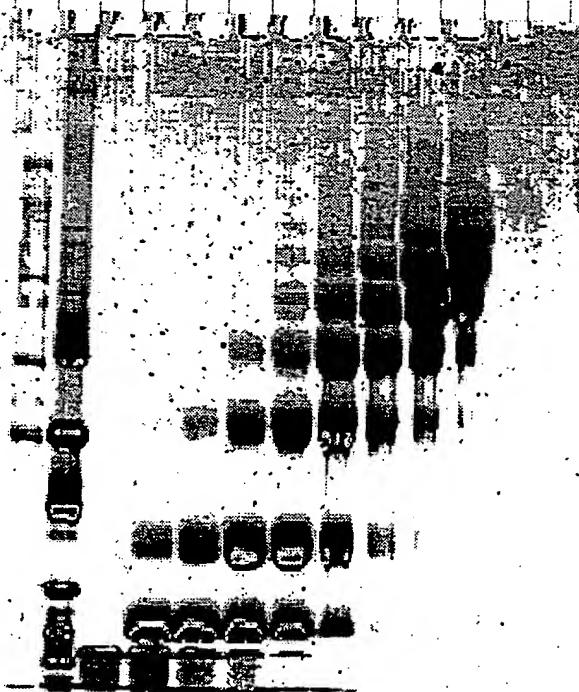
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
NT3	3	1.10	0.825	3.6	3.00
NT3 1X	4	2.00	1.50	4.0	6.00
NT3 2X	5	2.00	1.50	4.0	6.00
NT3 3X	6	2.00	1.50	4.0	6.00
NT3 4X	7	2.00	1.50	4.0	6.00
NT3 6X	8	2.00	1.50	4.0	6.00
NT3 8X	9	2.00	1.50	4.0	6.00
NT3 10X	10	2.00	1.50	4.0	6.00
NT3 12X	11	2.00	1.50	4.0	6.00



To Page No. X

Witnessed & Understood by me,

William Allen

Date

Invented by

Christine Farn

Date

Recorded by

Project No. 150103Book No. 5575

TITLE

Various Regulated CON-INF SDS PAGEFrom Page No. X

REDACTED

CON-INF-1

Date:

Operator: Chris

CON-INF-1

NB No.:

4-20% Gradient Mini Gel

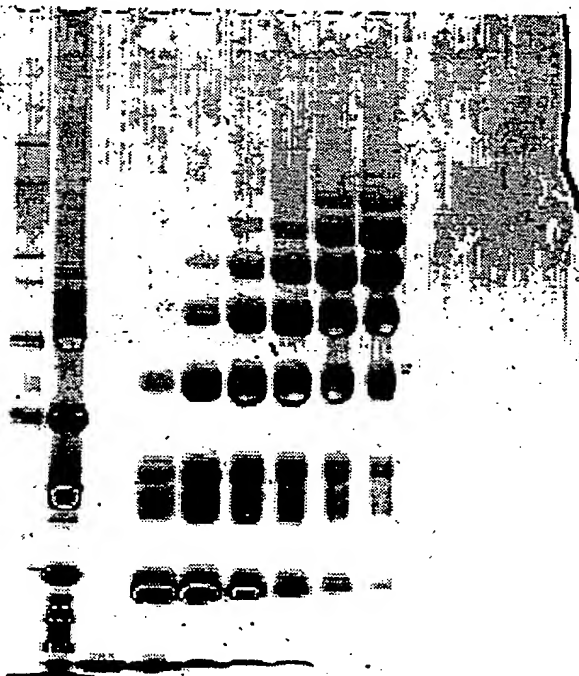
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
CON-INF	3	1.00	0.75	4.0	3.00
CON-INF 2X	4	2.00	1.50	4.0	6.00
CON-INF 4X	5	2.00	1.50	4.0	6.00
CON-INF 6X	6	2.00	1.50	4.0	6.00
CON-INF 8X	7	2.00	1.50	4.0	6.00
CON-INF 10X	8	2.00	1.50	4.0	6.00
CON-INF 12X	9	2.00	1.50	4.0	6.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farris

Date

Recorded by

From Page No. 1

REDACTED

GCSF-5

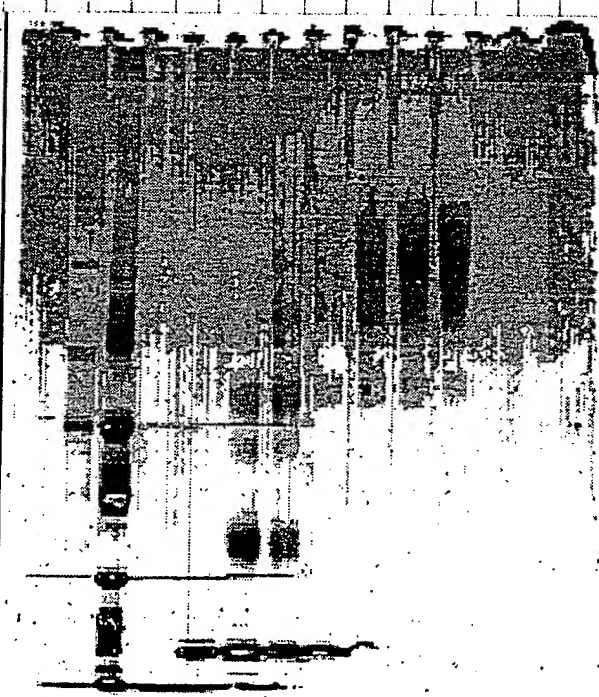
Date: _____
Operator: Chris
PEG-GCSF gel 1

NB No.: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
GCSF CONTROL	4	2.00	1.5	2.0	3.00
3X	5	2.00	1.50	4.0	6.00
6X	6	2.00	1.50	4.0	6.00
12X	7	2.00	1.50	4.0	6.00
18X	8	2.00	1.50	4.0	6.00
24X	9	2.00	1.50	4.0	6.00
36X	10	2.00	1.50	4.0	6.00



To Page No. 1

Witnessed & Understood by me,

William Callopan

Date

Invented by

Christine Jann

Date

Recorded by

From Page No. X

REDACTED

GCSF-6

Date: _____

Operator: Chris

PEG-GCSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

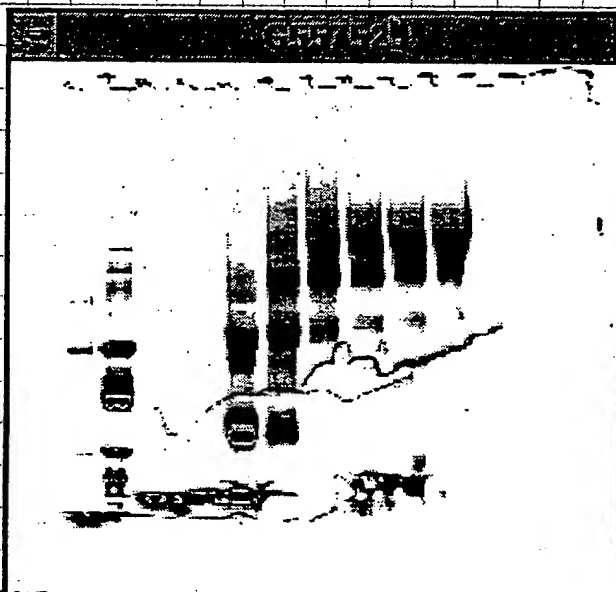
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
GCSF CONTROL	4	2.00	1.5	4.0	6.00
3X	5	2.00	1.50	15.0	22.50
6X	6	2.00	1.50	15.0	22.50
12X	7	2.00	1.50	15.0	22.50
18X	8	2.00	1.50	15.0	22.50
24X	9	2.00	1.50	15.0	22.50
36X	10	2.00	1.50	15.0	22.50

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Phu Thoi Gurn

Recorded by

Date

From Page No. X

REDACTED

GCSF-7

Date: _____

Operator: Chris

PEG-GCSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

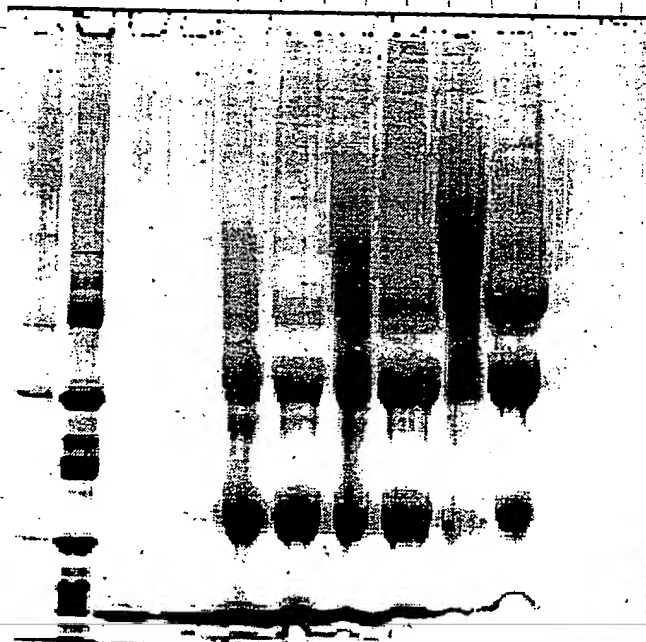
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
GCSF CONTROL	3	2.00	1.5	2.0	3.00
GCSF NH ₂ OH CONTROL	4	1.60	1.20	2.5	3.00
3X	5	2.00	1.50	13.3	20.00
3X NH ₂ OH	6	1.60	1.20	16.7	20.00
6X	7	2.00	1.50	13.3	20.00
6X NH ₂ OH	8	1.60	1.20	16.7	20.00
12X	9	2.00	1.50	13.3	20.00
12X NH ₂ OH	10	1.60	1.20	16.7	20.00



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Ferran

Recorded by

Date

From Page No. X

REDACTED

GCSF-8

Date: _____

Operator: Chris

PEG-GCSF gel 2

NB No.: _____

4-20% Gradient Mini Gel

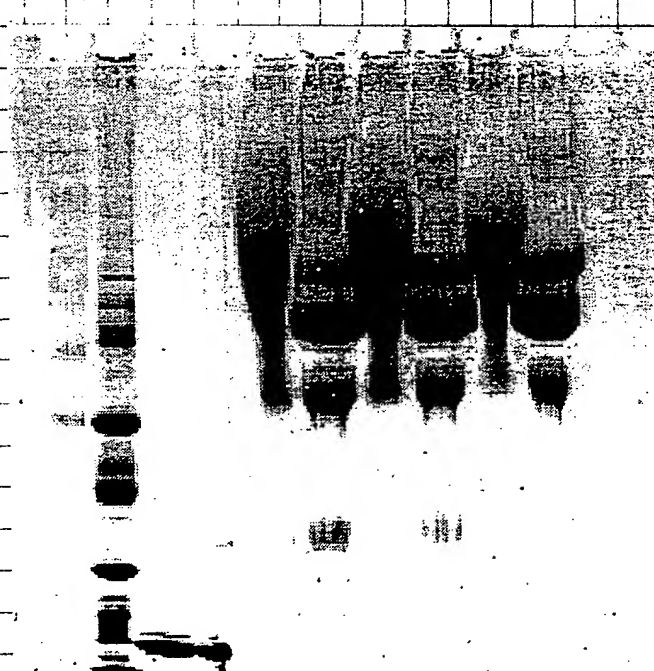
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			2	
LOW MW STD	2			5	
GCSF CONTROL	3	2.00	1.5	2.0	3.00
GCSF NH ₂ OH CONTROL	4	1.60	1.20	2.5	3.00
18X	5	2.00	1.50	13.3	20.00
18X NH ₂ OH	6	1.60	1.20	16.7	20.00
24X	7	2.00	1.50	13.3	20.00
24X NH ₂ OH	8	1.60	1.20	16.7	20.00
36X	9	2.00	1.50	13.3	20.00
36X NH ₂ OH	10	1.60	1.20	16.7	20.00



To Page No. _____

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farns

Recorded by

Date

TITLE *Yarrow Peg-OH GCSF SDS PAGE -1*From Page No. *X*

REDACTED

GCSF-7

Date: _____

Operator: Chris

PEG-GCSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

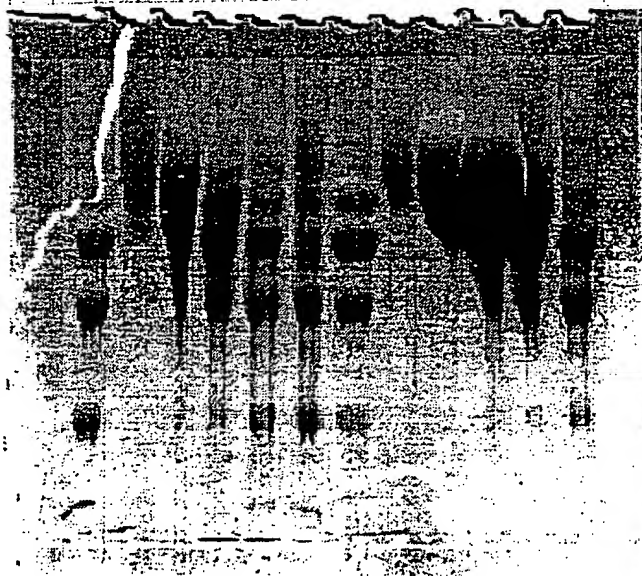
Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
12x NH2OH	1	2.00	1.50	6.7	10.00
12x FXN 3	2	0.76	0.57	17.7	10.00
12X FXN 19-20	3	0.59	0.45	22.4	10.00
12X FXN 21-22	4	1.08	0.81	12.4	10.00
12X FXN 24-25	5	1.78	1.34	7.5	10.00
12X FXN 27-28	6	0.56	0.42	23.6	10.00
18X NH2OH	7	2.00	1.50	6.7	10.00
18X FXN 2	8	0.93	0.70	14.3	10.00
18X FXN 10-11	9	0.43	0.32	24.6	8.00
18X FXN 12-14	10	0.78	0.59	17.0	10.00
18X FXN 15-16	11	0.56	0.42	23.8	10.00
18X FXN 18-19	12	0.42	0.31	25.7	8.00

To Page No. *X*

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrow

Recorded by

Date

From Page No. X

REDACTED

GCSF-8

Date: _____

Operator: Chris

PEG-GCSF gel 2

NB No.: _____

4-20% Gradient Mini Gel

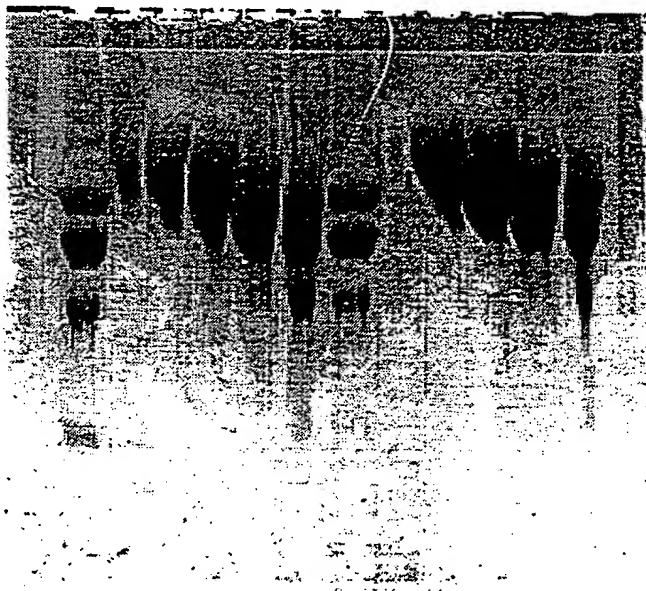
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
24X NH2OH	1	2.00	1.50	6.7	10.00
24x FXN 2-3	2	0.63	0.47	21.3	10.00
24X FXN 7-8	3	0.51	0.39	25.9	10.00
24X FXN 9-11	4	0.83	0.62	16.1	10.00
24X FXN 12-14	5	1.88	1.41	7.1	10.00
24X FXN 15-16	6	0.77	0.58	17.3	10.00
36X NH2OH	7	2.00	1.50	6.7	10.00
36X FXN 2-3	8	1.84	1.38	7.2	10.00
36X FXN 7-8	9	0.79	0.60	16.8	10.00
36X FXN 9-11	10	1.90	1.43	7.0	10.00
36X FXN 12-14	11	1.49	1.11	9.0	10.00
36X FXN 15-17	12	0.55	0.41	24.2	10.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date _____

Invented by _____

Recorded by _____

Date _____

From Page No. X

REDACTED

GCSF-9

Date: _____

Operator: Chris

PEG-GCSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

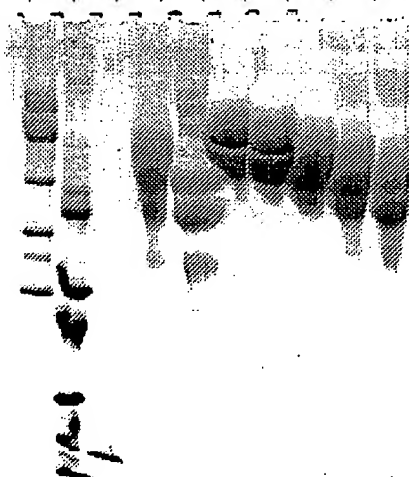
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			5.0	
LOW MW STD	2			5.0	
GCSF	3	1.00	0.75	4.0	3.00
PEG-GCSF 36X RXN	4	1.60	1.20	8.3	10.00
PEG-GCSF 36X NH ₂ OH	5	1.60	1.20	8.3	10.00
FXN 21-22	6	0.67	0.50	20.0	10.00
FXN 28-29	7	0.63	0.47	21.3	10.00
FXN 31-32	8	0.91	0.68	14.7	10.00
FXN 40-41	9	0.77	0.58	17.3	10.00
FXN 42-43	10	0.62	0.47	21.4	10.00



To Page No. X

Witnessed & Understood by me,

William Allston

Date

Invented by

Christine Farnan

Date

Recorded by

From Page No. X

REDACTED

BDNF-2

Date: _____

Operator: Chris

PEG-BDNF gel 1

NB No.: _____

4-20% Gradient Mini Gel

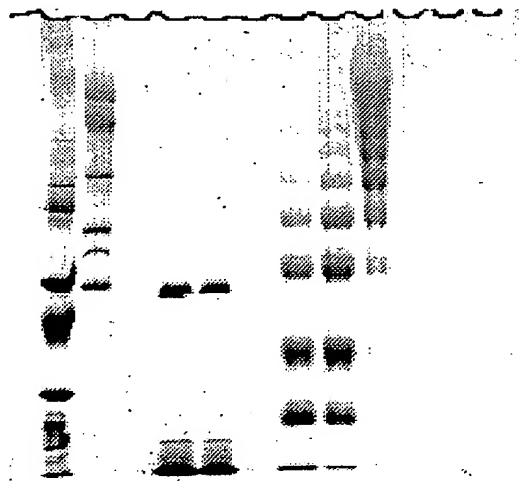
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
HI MW STD	1			4.0	
LOW MW STD	2			4.0	
BDNF START	4	2.20	1.65	1.8	3.00
BDNF CONTROL	5	2.00	1.50	2.0	3.00
PEG-OH BDNF 2X	7	1.00	0.75	8.0	6.00
PEG-OH BDNF 4X	8	1.00	0.75	8.0	6.00
PEG-OH BDNF 8X	9	1.00	0.75	8.0	6.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine J. Finn

Recorded by

Date

From Page No. 1

REDACTED

CON-INF-2

Date: _____

Operator: Chris

CON-INF-gel 1

NB No.: _____

4-20% Gradient Mini Gel

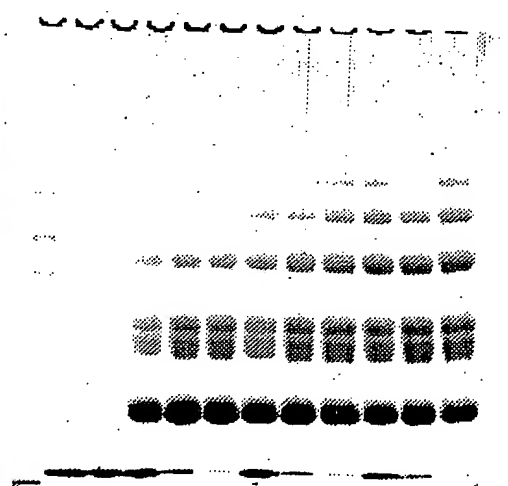
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			5.0	
CON INF START	2	0.20	0.15	20.0	3.00
0X CON-INF CONTROL	3	0.37	0.28	10.7	3.00
2X unpurified	4	2.00	1.50	4.0	6.00
2x purified pH 4.25	5	0.44	0.33	18.3	6.00
2x purified pH 3.50	6	0.35	0.26	22.9	6.00
4x unpurified	7	2.00	1.50	4.0	6.00
4x purified pH 4.25	8	0.42	0.31	19.1	6.00
4x purified pH 3.50	9	0.61	0.45	13.2	6.00
6x unpurified	10	2.00	1.50	4.0	6.00
6x purified pH 4.25	11	0.82	0.62	9.7	6.00
6x purified pH 3.50	12	0.83	0.62	9.7	6.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date _____

Invented by

Christine Farnon

Date _____

Recorded by _____

Project No. _____

Book No. 3575TITLE Various REG-CON INF SDS PAGE - 2From Page No. 4

REDACTED

CON-INF-3

Date: _____

Operator: Chris

CON-INF-gel 2

NB No.: _____

4-20% Gradient Mini Gel

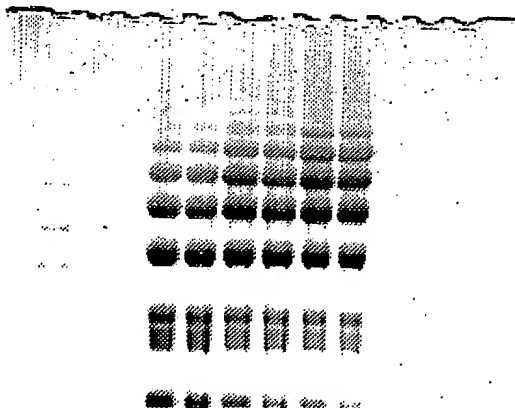
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			5.0	
CON INF START	2	0.20	0.15	20.0	3.00
0X CON-INF CONTROL	3	0.37	0.28	10.7	3.00
8X unpurified	4	2.00	1.50	4.0	6.00
8X purified pH 3.50	5	0.94	0.71	8.5	6.00
10X unpurified	6	2.00	1.50	4.0	6.00
10X purified pH 3.50	7	1.20	0.90	6.7	6.00
12x unpurified	8	2.00	1.50	4.0	6.00
12X purified pH 3.50	9	1.38	1.04	5.8	6.00



To Page No. _____

Witnessed & Understood by me,

Date

Invented by

Christine Tamm

Date

Recorded by

William Callahan

From Page No. X

REDACTED

GCSF-10

Date: _____

Operator: Chris

PEG-GCSF gel 1

NB No.: _____

4-20% Gradient Mini Gel

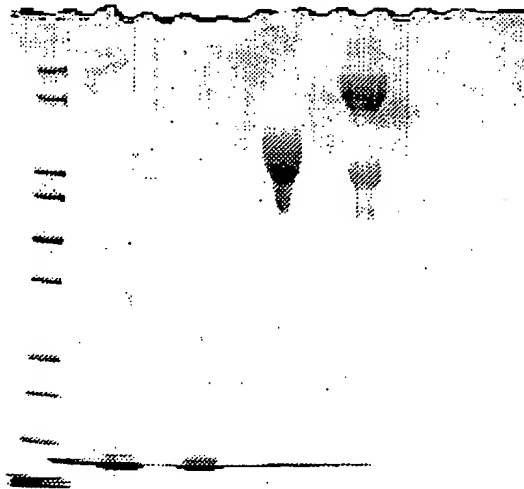
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD.	1			10.0	
GCSF	3	0.45	0.34	14.9	5.00
GCSF+NH ₂ OH	5	0.64	0.48	10.5	5.00
PEG-GCSF (fxn 28+29)	7	0.70	0.53	19.0	10.00
PEG-GCSF + NH ₂ OH	9	1.17	0.88	11.4	10.00



To Page No. X

Witnessed & Understood by me,

William Allahn

Date

Invented by

Christine Farnum

Recorded by

Date

Project No. 104003Book No. 5575TITLE Various PEG-SCF SDS PAGEFrom Page No. 1

REDACTED

SCF-1

Date: _____

Operator: Chris

PEG-SCF gel 2

NB No.: _____

4-20% Gradient Mini Gel

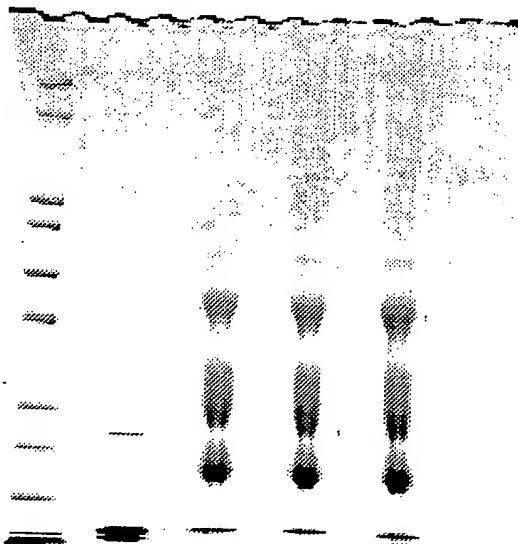
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MWSTD	1			10.0	
rrSCF CONT	3	2.20	1.65	3.0	5.00
5368-55	5	1.00	0.75	13.3	10.00
5001-84	7	1.00	0.75	13.3	10.00
4657-84	9	1.00	0.75	13.3	10.00

To Page No. X

Witnessed & Understood by me,

William Cellabon

Date

Invented by

Christine Farnsworth

Recorded by

Date

TITLE SCM-MPEG GCSF (Box) Various species ofProject No. 102003Book No. 5575

35

From Page No. X

REDACTED

GCSF-10

Date:

Operator: Chris

PEG-GCSF gel 1

NB No.:

4-20% Gradient Mini Gel

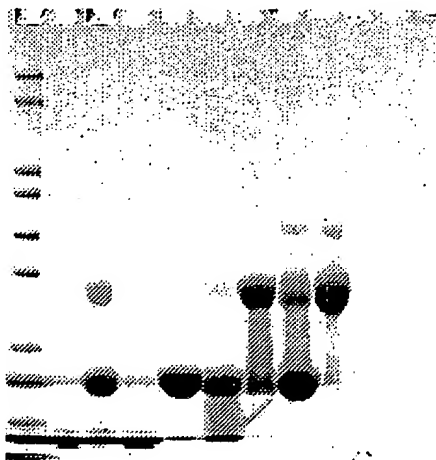
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF	2	1.00	0.75	4.0	3.00
PEG-GCSF 3.0X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	1.55	1.16	8.6	10.00
PEAK #2	5	0.65	0.49	20.5	10.00
PEAK #3	6	0.62	0.47	21.4	10.00
PEAK #4	7	1.24	0.93	10.7	10.00
PEAK #5	8	1.00	0.75	13.4	10.00
PEAK #6	9	0.84	0.63	15.9	10.00

To Page No. X

Witnessed & Understood by me,

William Allen

Date

Invented by

Christine Jones

Date

Recorded by

Project No. 102003Book No. 5515TITLE Various species of SCH-MPEG GCSF 1.5XFrom Page No. X

REDACTED

GCSF-11

Date:

Operator: Chris

PEG-GCSF gel 2

NB No.:

4-20% Gradient Mini Gel

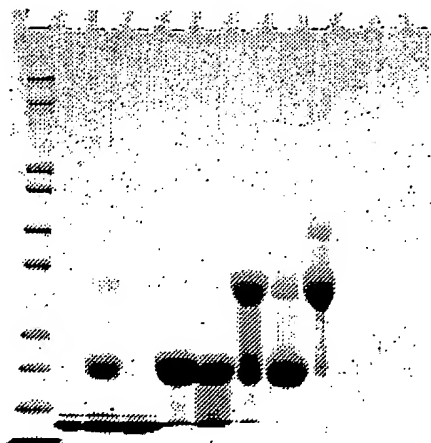
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF	2	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	1.51	1.14	8.8	10.00
PEAK #2	5	0.64	0.48	20.9	10.00
PEAK #3	6	1.04	0.78	12.8	10.00
PEAK #4	7	0.81	0.61	16.4	10.00
PEAK #5	8	0.92	0.69	14.6	10.00
PEAK #6	9	1.05	0.79	12.7	10.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farn

Date

Recorded by

From Page No. X

REDACTED

GCSF-12

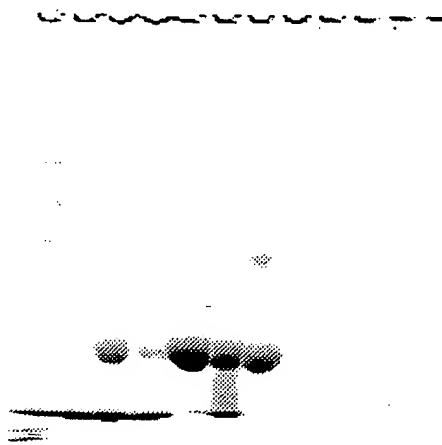
Date:
Operator: Chris
PEG-GCSF gel 1

NB No.:
4-20% Gradient Mini Gel

Running: Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF (start)	2	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	0.50	0.37	16.1	6.00
PEAK #2 (fxn 72)	5	1.12	0.84	11.9	10.00
PEAK #3 (fxn 62)	6	0.70	0.53	19.0	10.00
PEAK #4 (fxn 49)	7	0.45	0.34	29.8	10.00



To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Recorded by

Date

From Page No. 8

REDACTED

GCSF-13

Date:

Operator: Chris

PEG-GCSF gel 2

NB No.:

4-20% Gradient Mini Gel

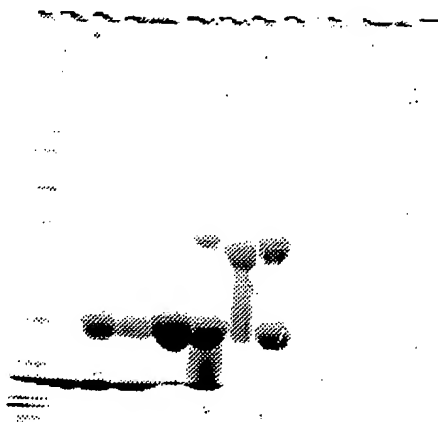
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF (start)	2	1.00	0.75	4.0	3.00
PEG-GCSF 3.0X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	0.63	0.48	21.0	10.00
PEAK #2 (fxn 71)	5	1.20	0.90	11.1	10.00
PEAK #3 (fxn 61)	6	0.59	0.44	22.5	10.00
PEAK #4 (fxn 51)	7	0.97	0.72	13.8	10.00
PEAK #5 (fxn 49)	8	1.60	1.20	8.3	10.00

To Page No. 8

Witnessed & Understood by me,

William Callahan

Date

Invented by

Recorded by

Date

From Page No. X

REDACTED

GCSF-14

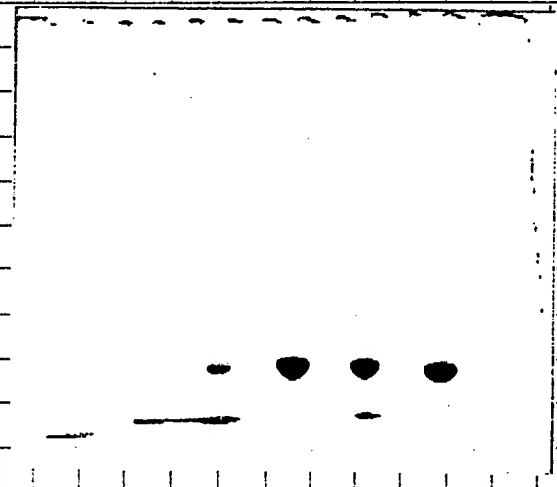
Date:
Operator: Chris
PEG-GCSF gel 1

NB No.:
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF (start)	3	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	5	2.00	1.50	6.7	10.00
PEAK #1	7	0.70	0.53	19.0	10.00
PEAK #2	9	0.89	0.67	15.0	10.00
PEAK #3	11	3.48	2.61	3.8	10.00



To Page No. X

Witnessed & Understood by me,
William Callahan

Date

Invented by

Christine Turner

Recorded by

Date

Project No. 102003Book No. 5375TITLE Scale-up Mono H-PEG-GCSF 1.5X SpeciesFrom Page No. X

REDACTED

PEG-GCSF (1)

Date:

Operator: Chris

NB No:

G-CSF gel 2

4-20% Gradient Mini Gel

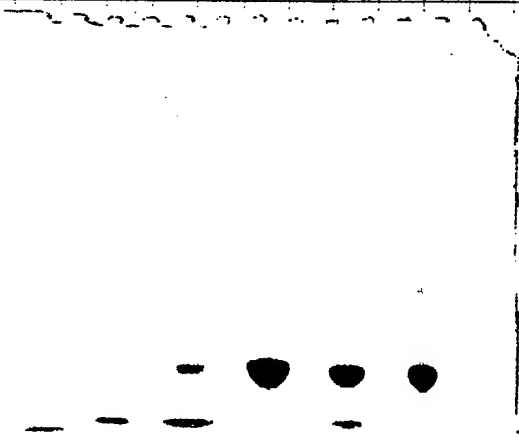
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF (START)	3	1.00	0.75	4.00	3.00
PEG-GCSF 1.5 RXN	5	2.00	1.50	6.67	10.00
PEAK #1A	7	4.53	3.40	2.95	10.00
PEAK #2A	9	2.48	1.86	5.38	10.00
PEAK #3A	11	0.94	0.70	14.22	10.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christina Johnson

Date

Recorded by

From Page No. X

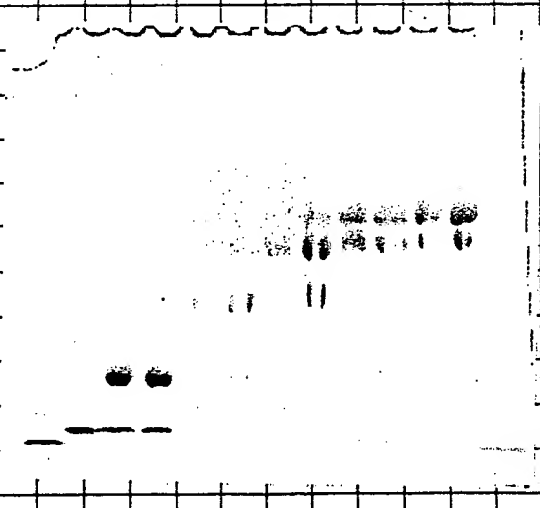
REDACTED

PEG-GCSF (2)

Date: _____
 Operator: Chris NB No: _____
 G-CSF gel 2 4-20% Gradient Mini Gel

Running Conditions
 constant current: 25 mA
 all non-reduced
 Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF std	2	1.00	0.75	4.00	3.00
4X RXN	3	1.90	1.43	7.02	10.00
4X IEX	4	0.81	0.81	16.38	10.00
8X RXN	5	1.90	1.43	7.02	10.00
8X IEX	6	2.07	1.55	6.44	10.00
12X RXN	7	1.90	1.43	7.02	10.00
12X IEX	8	1.68	1.26	7.94	10.00
16X RXN	9	1.90	1.43	7.02	10.00
16X IEX	10	2.00	1.50	6.67	10.00
20X RXN	11	1.90	1.43	7.02	10.00
20X IEX	12	1.60	1.20	8.33	10.00



To Page No. X

Witnessed & Understood by me, <u>William Allaha</u>	Date _____	Invented by <u>Christine Jones</u> Recorded by _____	Date _____
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Project No. 102003Book No. 5575TITLE Scale-up purified MOND4-PEG-GCSF Species

12

From Page No. X

REDACTED

PEG-GCSF

Date:

Operator: Chris

G-CSF gel 1

NB No:

4-20% Gradient Mini Gel

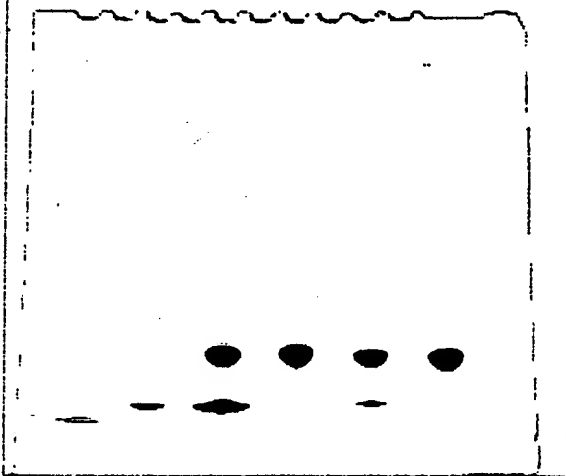
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF (START)	3	1.00	0.75	4.00	3.00
PEG-GCSF 1.5 RXN	5	2.00	1.50	6.67	10.00
PEAK #1A	7	0.57	0.42	23.57	10.00
PEAK #2A	9	0.55	0.42	24.03	10.00
PEAK #3A	11	0.57	0.43	23.46	10.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christian Fournier

Date

Recorded by

From Page No. X

REDACTED

PEG-GCSF

Date:

Operator: Chris

G-CSF gel 1

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF	3	1.00	0.75	4.00	3.00
PEG-GCSF 8X	5	3.00	2.25	4.44	10.00
PEG-GCSF 16X	7	3.00	2.25	4.44	10.00
PEG-GCSF 16X NEW	9	2.70	2.03	4.94	10.00

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Project No. 102003Book No. 5575TITLE DI PEG and Aggregate of 1.5x PEG-GCSFFrom Page No. X

REDACTED

PEG-GCSF

Date: _____

Operator: Chris

G-CSF gel 1

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF	3	1.00	0.75	4.00	3.00
RXN MIX	5	2.00	1.50	6.67	10.00
DI PEG-GCSF OF SEC	7	1.48	1.11	9.01	10.00
AGGRAGATE OF SEC	9	1.66	1.25	8.03	10.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Ferran

Date

Recorded by

From Page No. X

REDACTED

PEG-r-SCF

Date:

Operator: Chris

SCF gel 1

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
rrSCF lot#02103	3	0.88	0.66	7.58	5.00
PEG-rSCF #5368-55	5	1.00	0.75	13.33	10.00
3.75X RXN MIXTURE	7	2.00	1.50	6.67	10.00
4.25X RXN MIXTURE	9	2.00	1.50	6.67	10.00
4.75X RXN MIXTURE	11	2.00	1.50	6.67	10.00

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Collopy

Christine Farrar

Recorded by

Project No. 104003Book No. 5575TITLE Various lots of M-PEG- π SCFFrom Page No. 7

REDACTED

PEG-r-SCF

Date: _____

Operator: Chris

SCF gel 1

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
π SCF lot#02103	3	0.88	0.66	7.58	5.00
PEG- π SCF #6952-11	5	1.00	0.75	13.33	10.00
PEG- π SCF #5368-55	7	1.00	0.75	13.33	10.00
PEG- π SCF #5001-84	9	1.00	0.75	13.33	10.00
PEG- π SCF #4657-84	11	1.00	0.75	13.33	10.00

To Page No. 7

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Zaman

Date

Recorded by

TITLE pH 4.5-6 Horz. IEF Gel of MONO-PEG ACSF Species Book No. 5575

From Page No. X

REDACTED

Ampholine[®] PAGplate

Experimental result form

LKB

pH range 4.5-6.0

Anodic Electrode Solution 0.1M H₃PO₄

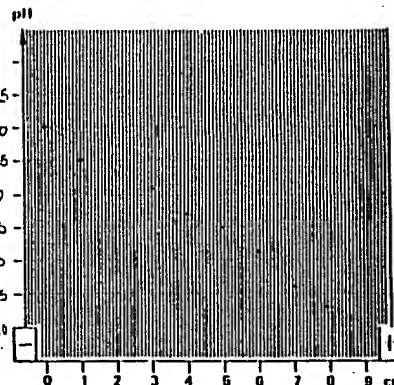
Cathodic Electrode Solution 0.1M NaOH

Date

Experiment No. Notebook No. 5575-47

Operator CCE

Sample No.	Sample description	Conc. (mg/ml)	Volume (μl)	Position	1	2	3	4	5	6	7
1	IEF Standards 2.5-6.9	1	10	✓							
2	SCF	1	10	✓							
3	GCSE	1	10	✓							
4	MONO PEG 1	.5	20	✓							
5	" 2	.5	20	✓							
6	" 3	.5	20	✓							
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											



Electrofocusing data

Cooling temperature

15 °C

pH measured at

4.5-6.0 °C

Time	Voltage	Current	Power
Start	690 V	19.4 mA	10 W
2:30	900 V	10.4 mA	10 W
3:00	1100 V	8.7 mA	10 W
3:30	1300 V	7.4 mA	10 W
4:00	1467 V	6.9 mA	10 W
4:30	1500 V	6.7 mA	10 W
End 5:00	1515 V	6.6 mA	10 W

05 50 0913

6 5 4 3 2 1

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christina Jordan

Recorded by

Date

From Page No. X

REDACTED

PEG-GCSF

Date:

Operator: Chris

G-CSF gel 1

NB No:

3-10 VERTICAL NON RED/IEF

Running Conditions

constant voltage: 200:400V

all non-reduced

Comassie BIORAD Method

Sample Code	Lane No.	Conc (mg/ml)	64% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF T6702	3	1.00	0.64	7.81	5.00
mono peg-g 6951-23	5	0.50	0.32	21.88	7.00
mono peg-g 6951-24	7	0.50	0.32	21.88	7.00
mono peg-g 6951-25	9	0.50	0.32	21.88	7.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Jones

Date

Recorded by

From Page No. 1

REDACTED

PEG-r-SCF

Date: _____
Operator: Chris
SCF gel 1

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
rrSCF lot#02103	3	0.88	0.66	7.58	5.00
PEG-rrSCF #6952-11	5	1.00	0.75	13.33	10.00
PEG-rrSCF #5368-55	7	1.00	0.75	13.33	10.00
PEG-rrSCF #5001-84	9	1.00	0.75	13.33	10.00
PEG-rrSCF #5001-84	11	1.00	0.75	13.33	10.00

To Page No. X

Witnessed & Understood by me,
William Callahan

Date

Invented by
Christine Jansen
Recorded by

Date

Project No. 104005Book No. 507TITLE pH 3-9 Vert IEF Bl Analysis of PEG-rSCF lot #5001-84From Page No. 1

REDACTED

PEG-r-SCF

Date:

Operator: Chris

SCF gel 1

NB No:

pH 3-9 Gradient Vertical IEF Gel

Running Conditions

constant voltage: 200V & 400V

all non-reduced

Comassie: ISS METHOD

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
STD	1			10.00	
rSCF lot#02103	3	0.88	0.44	11.36	5.00
PEG-rSCF #6952-11	5	1.00	0.50	20.00	10.00
PEG-rSCF #5368-55	7	1.00	0.50	20.00	10.00
PEG-rSCF #5001-84	9	1.00	0.50	20.00	10.00
used #5001-84	11	1.00	0.50	20.00	10.00

To Page No. 1

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Recorded by

From Page No. X

REDACTED

Ampholine[®] PAGplate

Experimental result form



pH range 4-7

Anode Electrode Solution 1M H₃PO₄

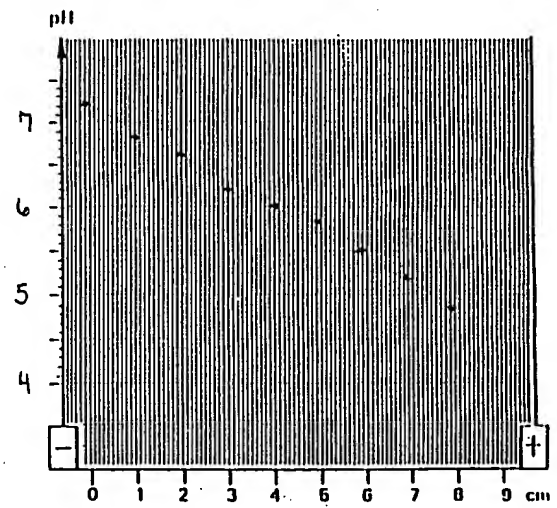
Cathode Electrode Solution 2M NaOH

Date

Experiment No.

Operator CF

Sample No.	Sample description	Conc. (mg/ml)	Volume (ul)	Position						
				1	2	3	4	5	6	7
8.1	STD	-	20							
9.2	SCF lot # 02103	188	8.8							
11.3	PEG-trSCF lot # 6992-11	1	20							
12.4	PEG-trSCF lot # 5368-55	1	20							
13.6	PEG-trSCF lot # 5001-84	1	20							
14.6	used PEG-trSCF lot # 5001-84	1	20							
7										
8										
9										
10										
11										
12										
13										
14										
15										
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18										
19										
20										
21										
22										
23										
24										



Electrofocusing data

Cooling temperature 15 °C

pH measured at 15 °C

Time	Voltage	Current	Power
START 10:00	380V	26mA	10W
11:30	1000V	9mA	10W
11:00	1100V	6mA	6W
11:30	1200V	5mA	6W
13:00	1350V	4.8mA	6W
12:30	1300V	4.5mA	6W
END 1:00	1550V	4.5mA	6W

95 50 0913

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Recorded by

To Page No. X

Project No. 102003

Book No. 5515

TITLE Western Blot of Mono-PEG-GCSF sera samples

From Page No. 1

REDACTED

PEG-GCSF

Date:

Operator: Chris

PEG-GCSF study 021593

t=5day sera samples

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

Western Blot SOP (polyclonal rabbit anti-GCSF development)

Sample Code	Lane No.	Conc (ng/ml)	80% of Conc (ng/ml)	Load uL	Load ug
prestained mw markers	1			10	
NTERM 6951-23	2	10.00	8.00	3	25.00
LYS 35 6951-24	3	10.00	8.00	3	25.00
GRP 2 ANIMAL 2	4			25	
GRP 2 ANIMAL 3	5			25	
GRP 3 ANIMAL 1	6			25	
GRP 3 ANIMAL 2	7			25	
GRP 3 ANIMAL 3	8			25	
GRP 4 ANIMAL 1	9			25	
GRP 4 ANIMAL 2	10			25	
GRP 5 ANIMAL 1	11			25	
GRP 5 ANIMAL 2	12			25	

(Unsuccessful - no copy)

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnsworth

Date

Recorded by

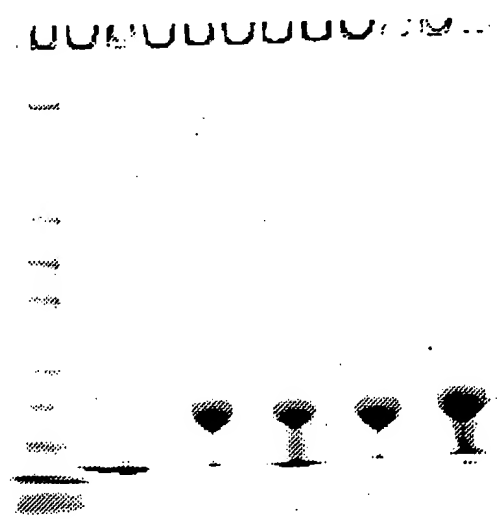
From Page No. 2

REDACTED PEG-GCSF

Date: Operator: Chris PEG-GCSF NB No: 4-20% Gradient Mini Gel

Running Conditions constant current: 25 mA all non-reduced 4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	80% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			10	
GCSF T6702	3	1.00	0.80	6	5.00
NTERM 6951-23	5	0.50	0.40	25	10.00
LYS 35 6951-24	7	0.50	0.40	25	10.00
LYS 41 6951-25	9	0.50	0.40	25	10.00
LYS 35 PURIFIED	11	0.50	0.40	25	10.00



To Page No. 1

Witnessed & Understood by me, William Collier	Date	Invented by Christine Jones	Date
		Recorded by	

From Page No. 2

REDACTED

PEG-GCSF-1

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

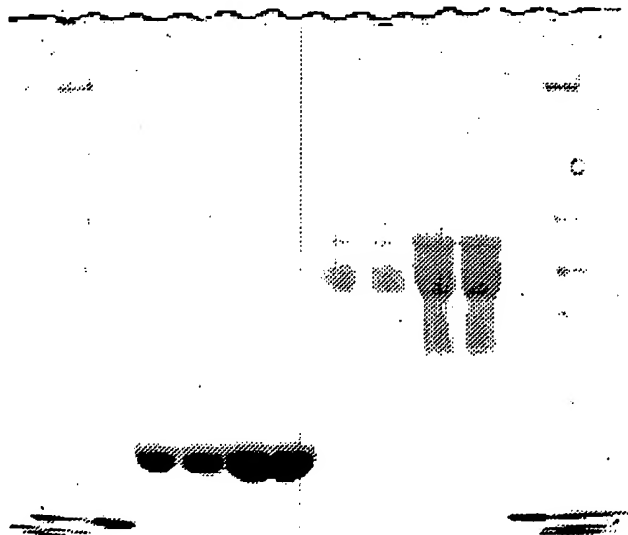
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM	3	1.00	0.75	6.7	5.00
N-TERM	4	1.00	0.75	6.7	5.00
N-TERM	5	1.00	0.75	13.3	10.00
N-TERM	6	1.00	0.75	13.3	10.00
TRI-TETRA	7	1.00	0.75	6.7	5.00
TRI-TETRA	8	1.00	0.75	6.7	5.00
TRI-TETRA	9	1.00	0.75	13.3	10.00
TRI-TETRA	10	1.00	0.75	13.3	10.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MW STD	12			15	



To Page No. 2

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 1

REDACTED

PEG-GCSF-2

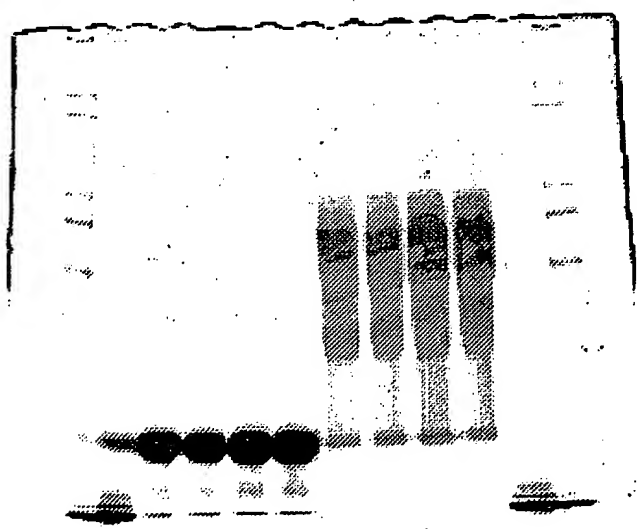
Date: _____
Operator: Chris
PEG-GCSF

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.7	2.00
N-TERM	3	1.00	0.75	2.7	2.00
N-TERM	4	1.00	0.75	2.7	2.00
N-TERM	5	1.00	0.75	5.3	4.00
N-TERM	6	1.00	0.75	5.3	4.00
TRI-TETRA	7	1.00	0.75	2.7	2.00
TRI-TETRA	8	1.00	0.75	2.7	2.00
TRI-TETRA	9	1.00	0.75	5.3	4.00
TRI-TETRA	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.7	2.00
MW STD	12			15	



To Page No. 1

Witnessed & Understood by me,
William Callahan

Date

Invented by Christina Furman
Recorded by

Date

From Page No. 2

PEG-GCSF WEST

REDACTED

Date: _____

Operator: Chris

PEG-GCSF study G061493

T=1 day sera samples

NB No: _____

4-20% Gradient Mini Gel

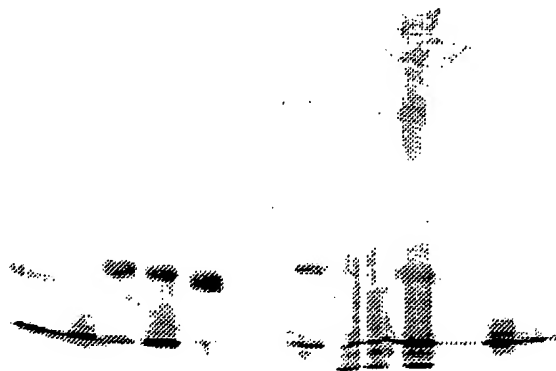
Running Conditions

constant current: 25 mA

all non-reduced

Western Blot SOP (polyclonal rabbit anti-GCSF development)

Sample Code	Lane No.	Conc (ng/ul)	80% of Conc (ng/ul)	Load uL	Load ng
MW STD	1			15.0	
GCSF T6702	2	50.0	40.0	3.0	120.0
NTERM 6951-23	3	50.0	40.0	10.0	400.0
LYS 35 6951-24	4	50.0	40.0	10.0	400.0
LYS 41 6951-25	5	50.0	40.0	10.0	400.0
GCSF T=1.5	7	3.0	2.4	28.0	67.2
NTERM T=1	8	3.0	2.4	28.0	67.2
LYS 35 T=1	9	3.0	2.4	28.0	67.2
LYS 41 T=1	10	3.0	2.4	28.0	67.2
GCSF T6702	12	50.0	40.0	3.0	120.0

To Page No. 2

Witnessed & Understood by me,

William Allen

Date

Invented by

Christina Farnan

Recorded by

Date

From Page No. X

PEG-GCSF-1

REDACTED

Date: _____
Operator: Chris
PEG-GCSF

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM 4C	3	1.00	0.75	6.7	5.00
N-TERM 4C	4	1.00	0.75	13.3	10.00
N-TERM 45C	5	1.00	0.75	6.7	5.00
N-TERM 45C	6	1.00	0.75	13.3	10.00
TRI-TETRA 4C	7	1.00	0.75	6.7	5.00
TRI-TETRA 4C	8	1.00	0.75	13.3	10.00
TRI-TETRA 45C	9	1.00	0.75	6.7	5.00
TRI-TETRA 45C	10	1.00	0.75	13.3	10.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MW STD	12			15	



To Page No. X

Witnessed & Understood by me, <u>William Callahan</u>	Date	Invented by <u>Christine Tarran</u>	Date
		Recorded by	

Project No. 102003
Book No. 5515TITLE SOS PAGE Analysis for MPGFE1 / SilverFrom Page No. 4

REDACTED

PEG-GCSF-2

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

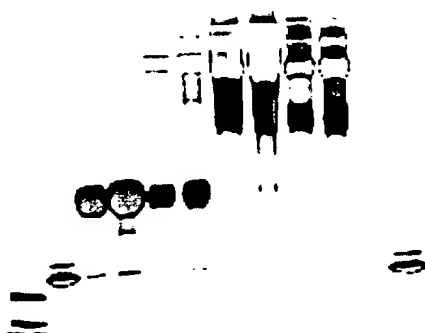
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
N-TERM 4C	3	1.00	0.75	2.7	2.00
N-TERM 4C	4	1.00	0.75	5.3	4.00
N-TERM 45C	5	1.00	0.75	2.7	2.00
N-TERM 45C	6	1.00	0.75	5.3	4.00
TRI-TETRA 4C	7	1.00	0.75	2.7	2.00
TRI-TETRA 4C	8	1.00	0.75	5.3	4.00
TRI-TETRA 45C	9	1.00	0.75	2.7	2.00
TRI-TETRA 45C	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	

To Page No. 4

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine J. Janssen

Recorded by

Date

From Page No. X

PEG-GCSF-1

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MWSTD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM 4C	3	1.00	0.75	6.7	5.00
N-TERM 4C	4	1.00	0.75	13.3	10.00
N-TERM 45C	5	1.00	0.75	6.7	5.00
N-TERM 45C	6	1.00	0.75	13.3	10.00
TRI-TETRA 4C	7	1.00	0.75	6.7	5.00
TRI-TETRA 4C	8	1.00	0.75	13.3	10.00
TRI-TETRA 45C	9	1.00	0.75	6.7	5.00
TRI-TETRA 45C	10	1.00	0.75	13.3	10.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MWSTD	12			15	

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Forner

Recorded by

Date

Project No. 102603
Book No. 50B

TITLE SOS PAGE Analysis for MPG

F2/Silver

From Page No. X

PEG-GCSF-2

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
N-TERM 4C	3	1.00	0.75	2.7	2.00
N-TERM 4C	4	1.00	0.75	5.3	4.00
N-TERM 45C	5	1.00	0.75	2.7	2.00
N-TERM 45C	6	1.00	0.75	5.3	4.00
TRI-TETRA 4C	7	1.00	0.75	2.7	2.00
TRI-TETRA 4C	8	1.00	0.75	5.3	4.00
TRI-TETRA 45C	9	1.00	0.75	2.7	2.00
TRI-TETRA 45C	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Jansen

Date

Recorded by

From Page No. X

PEG-GCSF-3

REDACTED

Date:
Operator: Chris
PEG-GCSF

NB No:
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/comassie and silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
GCSF IN SORB.	3	1.00	0.75	2.0	1.50
GCSF IN SORB.	4	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWEEN	5	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWEEN	6	1.00	0.75	4.0	3.00
GCSF IN SORB.	7	1.00	0.75	1.0	0.75
GCSF IN SORB.	8	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWEEN	9	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWEEN	10	1.00	0.75	2.0	1.50
GCSF T6702	11	1.00	0.75	1.0	0.75
MW STD	12			10	

To Page No. X

Witnessed & Understood by me, <i>Villem Calabro</i>	Date	Invented by <i>Christina Jaron</i>	Date
		Recorded by	

Project No. 102003Book No. 5575PAGE
TITLE SDS Analysis of MPG

T=3 / Coomassie

From Page No. X

REDACTED

PEG-GCSF-1

Date: _____

Operator: Chris

PEG-GCSF

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/comassie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM 4C	3	1.00	0.75	6.7	5.00
N-TERM 4C	4	1.00	0.75	13.3	10.00
N-TERM 45C	5	1.00	0.75	6.7	5.00
N-TERM 45C	6	1.00	0.75	13.3	10.00
TRI-TETRA 4C	7	1.00	0.75	6.7	5.00
TRI-TETRA 4C	8	1.00	0.75	13.3	10.00
TRI-TETRA 45C	9	1.00	0.75	6.7	5.00
TRI-TETRA 45C	10	1.00	0.75	13.3	10.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MW STD	12			15	

To Page No. X

Witnessed & Understood by me,

William Allerton

Date

Invented by

Recorded by

Date

From Page No. 2

REDACTED

PEG-GCSF-2

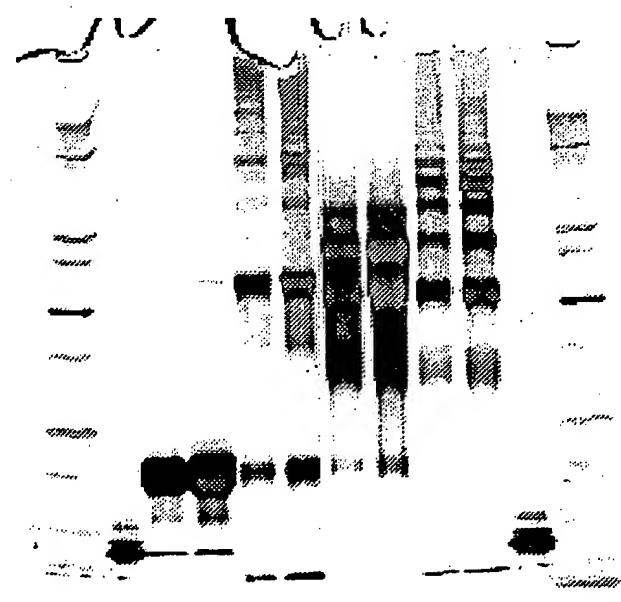
Date: _____
 Operator: Chris
 PEG-GCSF

NB No: _____
 4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
 all non-reduced
 4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
N-TERM 4C	3	1.00	0.75	2.7	2.00
N-TERM 4C	4	1.00	0.75	5.3	4.00
N-TERM 45C	5	1.00	0.75	2.7	2.00
N-TERM 45C	6	1.00	0.75	5.3	4.00
TRI-TETRA 4C	7	1.00	0.75	2.7	2.00
TRI-TETRA 4C	8	1.00	0.75	5.3	4.00
TRI-TETRA 45C	9	1.00	0.75	2.7	2.00
TRI-TETRA 45C	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	



To Page No. 2

Witnessed & Understood by me, <i>William Collette</i>	Date	Invented by <i>Christine Turner</i>	Date
		Recorded by	

From Page No. X

REDACTED

PEG-GCSF-3

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

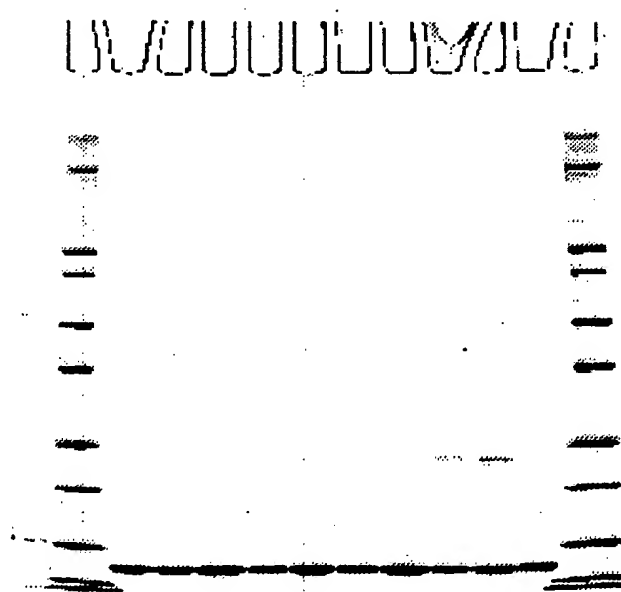
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/comassie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	3	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	4	1.00	0.75	4.0	3.00
GCSF IN SORB. 45C	5	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	6	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	4.0	3.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	



To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Horan

Recorded by**Date**

From Page No. X

REDACTED

PEG-GCSF-4

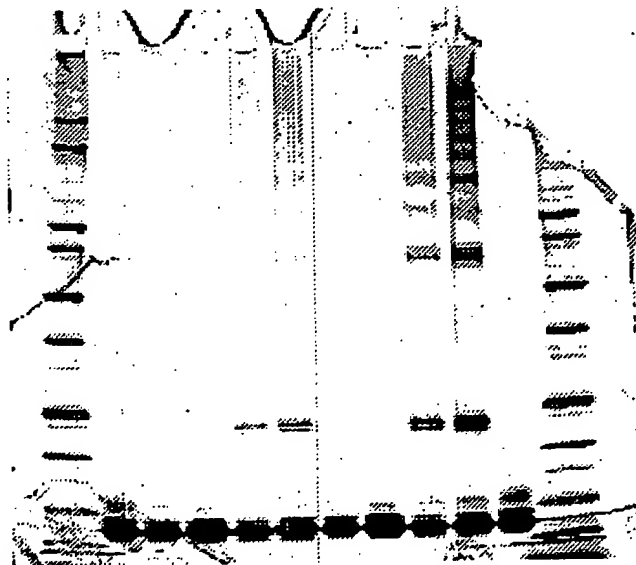
Date: _____
 Operator: Chris
 PEG-GCSF

NB No: _____
 4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
 all non-reduced **Silver**
 4-20% Gradient Mini Gel/**Silver** SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			10	
GCSF T6702	2	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	3	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	4	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	5	1.00	0.75	1.0	0.75
GCSF IN SORB. 45C	6	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	2.0	1.50
GCSF T6702	11	1.00	0.75	1.0	0.75
MW STD	12			10	



To Page No. X

Witnessed & Understood by me,

William Collier

Date

Invented by

Christine Farn

Date

Recorded by

Project No. 107003Book No. 5575TITLE SOS PAGE of 2X Purified Serum Samples from G:From Page No. X

PEG-GCSF WEST

REDACTED

Date: _____

Operator: Chris

PEG-GCSF study G061493

T=1day sera samples

NB No: _____

4-20% Gradient Mini Gel

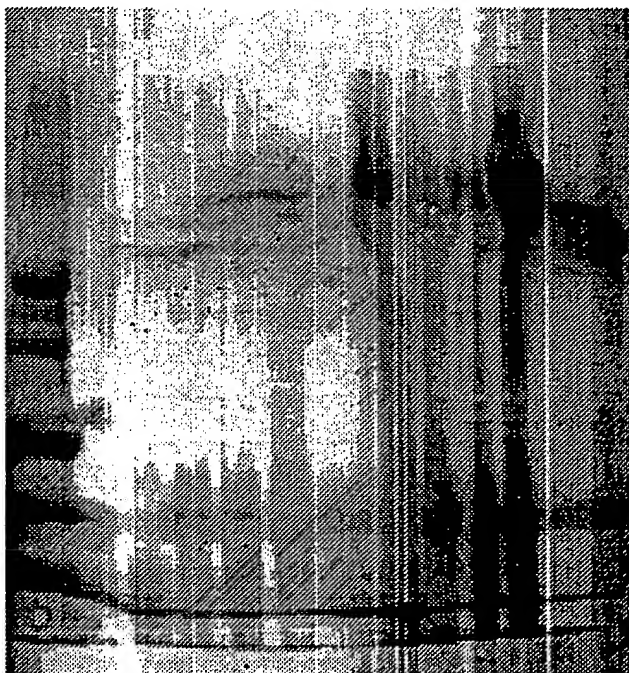
Running Conditions

constant current: 25 mA

all non-reduced

Western Blot SOP (polyclonal rabbit anti-GCSF development)

Sample Code	Lane No.	Conc (ng/ul)	80% of Conc (ng/ul)	Load uL	Load ng
MW STD	1			10.0	
GCSF T6702	3	500.0	400.0	1.0	400.0
NTERM 6951-23	4	500.0	400.0	1.0	400.0
LYS 35 6951-24	5	500.0	400.0	1.0	400.0
LYS 41 6951-25	6	500.0	400.0	1.0	400.0
GCSF T=1.5	8	3.0	2.4	28.0	67.2
NTERM T=1	9	3.0	2.4	28.0	67.2
LYS 35 T=1	10	3.0	2.4	28.0	67.2
LYS 41 T=1	11	3.0	2.4	28.0	67.2

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William CallahanChristine Farrar

Recorded by

From Page No. X

PEG-GCSF-1

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

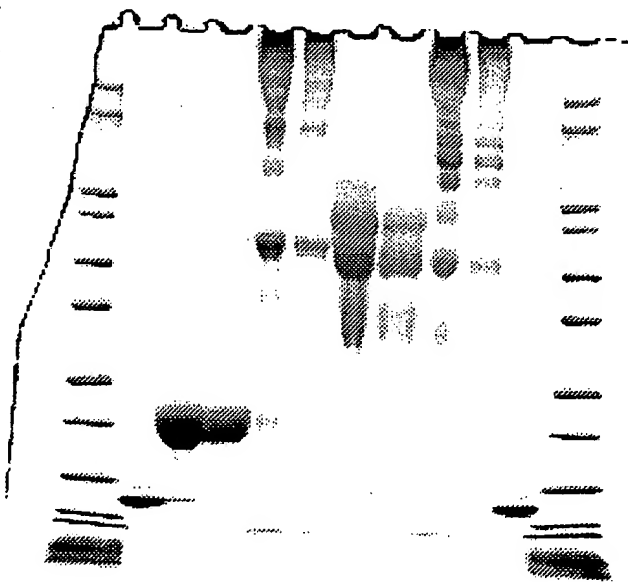
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM 4C	3	1.00	0.75	13.3	10.00
N-TERM 4C	4	1.00	0.75	6.7	5.00
N-TERM 45C	5	1.00	0.75	13.3	10.00
N-TERM 45C	6	1.00	0.75	6.7	5.00
TRI-TETRA 4C	7	1.00	0.75	13.3	10.00
TRI-TETRA 4C	8	1.00	0.75	6.7	5.00
TRI-TETRA 45C	9	1.00	0.75	13.3	10.00
TRI-TETRA 45C	10	1.00	0.75	6.7	5.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MW STD	12			15	

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnan

Date

Recorded by

Project No. 102003
Book No. 5575TITLE SDS PAGE Analysis for MPT=4/SilverFrom Page No. X

PEG-GCSF-2

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

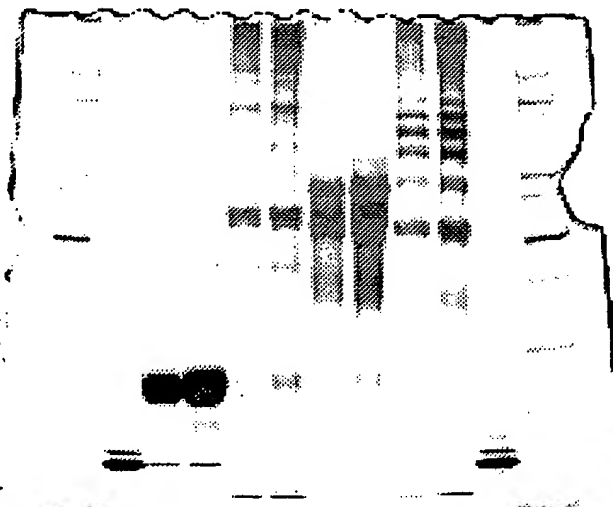
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
N-TERM 4C	3	1.00	0.75	2.7	2.00
N-TERM 4C	4	1.00	0.75	5.3	4.00
N-TERM 45C	5	1.00	0.75	2.7	2.00
N-TERM 45C	6	1.00	0.75	5.3	4.00
TRI-TETRA 4C	7	1.00	0.75	2.7	2.00
TRI-TETRA 4C	8	1.00	0.75	5.3	4.00
TRI-TETRA 45C	9	1.00	0.75	2.7	2.00
TRI-TETRA 45C	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrar

Date

Recorded by

From Page No. 1

PEG-GCSF-3

REDACTED

Date: _____

Operator: Chris

PEG-GCSF

NB No: _____

4-20% Gradient Mini Gel

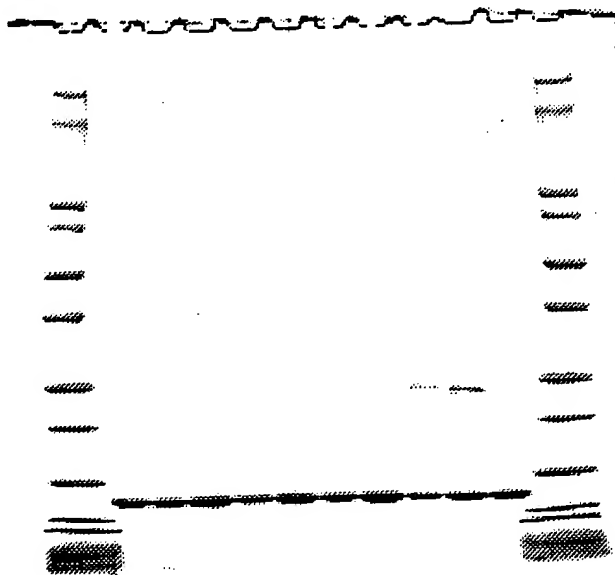
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/comassie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	3	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	4	1.00	0.75	4.0	3.00
GCSF IN SORB. 45C	5	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	6	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	4.0	3.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	

To Page No. 1

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farnon

Recorded by

Date

From Page No. 4

PEG-GCSF-4

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

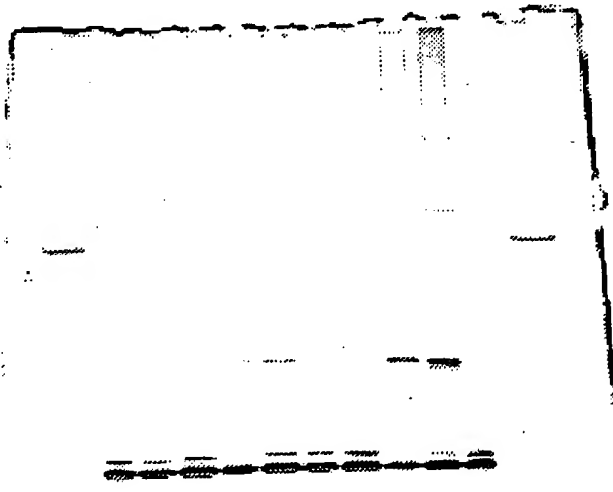
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel ^{Silver} ~~Leucine~~ SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			10	
GCSF T6702	2	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	3	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	4	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	5	1.00	0.75	1.0	0.75
GCSF IN SORB. 45C	6	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	2.0	1.50
GCSF T6702	11	1.00	0.75	1.0	0.75
MW STD	12			10	



To Page No. 4

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Christine Farnsworth

Recorded by

From Page No. 4

PEG-GCSF-1

REDACTED

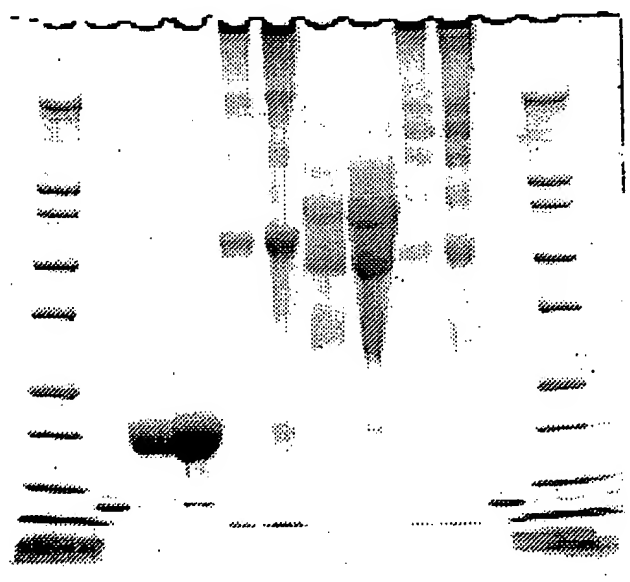
Date:
Operator: Chris
PEG-GCSF

NB No:
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	4.0	3.00
N-TERM 4C	3	1.00	0.75	13.3	10.00
N-TERM 4C	4	1.00	0.75	6.7	5.00
N-TERM 45C	5	1.00	0.75	13.3	10.00
N-TERM 45C	6	1.00	0.75	6.7	5.00
TRI-TETRA 4C	7	1.00	0.75	13.3	10.00
TRI-TETRA 4C	8	1.00	0.75	6.7	5.00
TRI-TETRA 45C	9	1.00	0.75	13.3	10.00
TRI-TETRA 45C	10	1.00	0.75	6.7	5.00
GCSF T6702	11	1.00	0.75	4.0	3.00
MW STD	12			15	



To Page No. X

Witnessed & Understood by me, <i>Nilemini Callalan</i>	Date	Invented by <i>Chaitanya Farnan</i>	Date
		Recorded by	

From Page No. 1

PEG-GCSF-2

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

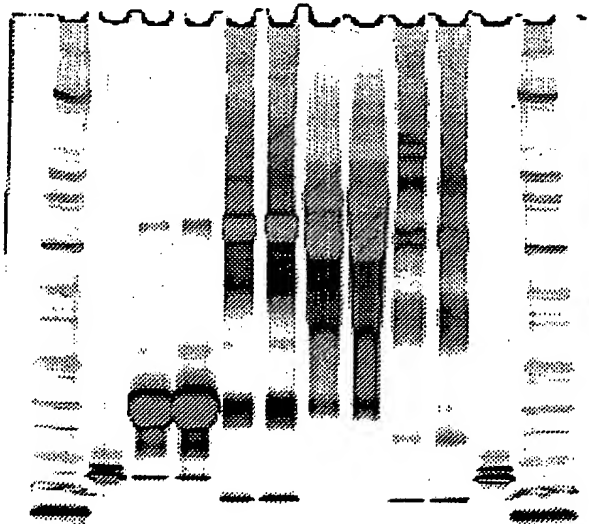
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/silver stain SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
N-TERM 4C	3	1.00	0.75	2.7	2.00
N-TERM 4C	4	1.00	0.75	5.3	4.00
N-TERM 45C	5	1.00	0.75	2.7	2.00
N-TERM 45C	6	1.00	0.75	5.3	4.00
TRI-TETRA 4C	7	1.00	0.75	2.7	2.00
TRI-TETRA 4C	8	1.00	0.75	5.3	4.00
TRI-TETRA 45C	9	1.00	0.75	2.7	2.00
TRI-TETRA 45C	10	1.00	0.75	5.3	4.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	

To Page No. 1

Witnessed & Understood by me,

Date

Invented by

Date

William C. LlopisChristine Farni

Recorded by

TITLE SDS PAGE Analysis for GCSF @45°C T=3/Comassie

Book No. 5570

From Page No. X

PEG-GCSF-3

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

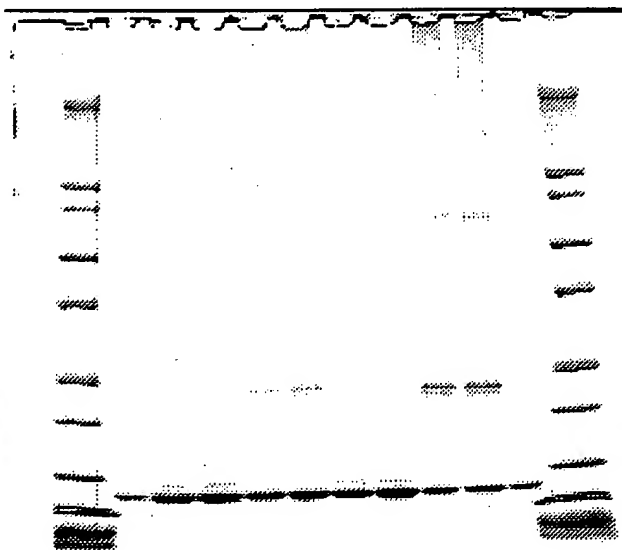
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/comassie SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			15	
GCSF T6702	2	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	3	1.00	0.75	2.0	1.50
GCSF IN SORB. 4C	4	1.00	0.75	4.0	3.00
GCSF IN SORB. 45C	5	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	6	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	4.0	3.00
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	4.0	3.00
GCSF T6702	11	1.00	0.75	2.0	1.50
MW STD	12			15	



To Page No. X

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

William Callahan

Christine Farnon

From Page No. 4

PEG-GCSF-4

REDACTED

Date: _____

Operator: Chris

PEG-GCSF

NB No: _____

4-20% Gradient Mini Gel

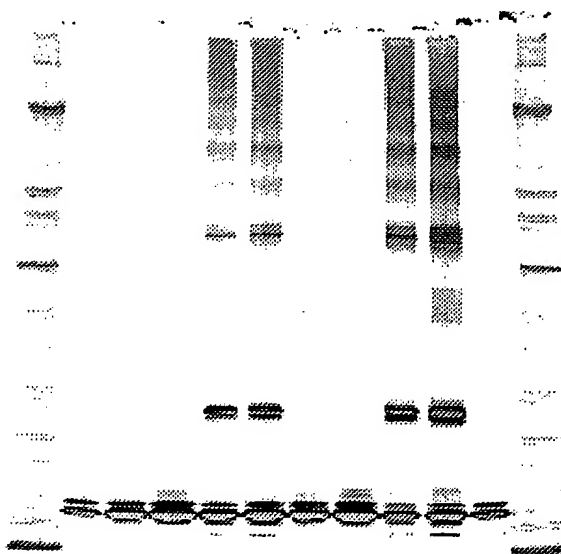
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/SILVER SOP

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			10	
GCSF T6702	2	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	3	1.00	0.75	1.0	0.75
GCSF IN SORB. 4C	4	1.00	0.75	2.0	1.50
GCSF IN SORB. 45C	5	1.00	0.75	1.0	0.75
GCSF IN SORB. 45C	6	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 4C	7	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 4C	8	1.00	0.75	2.0	1.50
GCSF IN SORB. W/TWN. 45C	9	1.00	0.75	1.0	0.75
GCSF IN SORB. W/TWN. 45C	10	1.00	0.75	2.0	1.50
GCSF T6702	11	1.00	0.75	1.0	0.75
MW STD	12			10	

To Page No. 4

Witnessed & Understood by me,

Date

William Callahan

Invented by

Christina Tarnai

Recorded by

Date

From Page No. 1

PEG-GCSF-

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

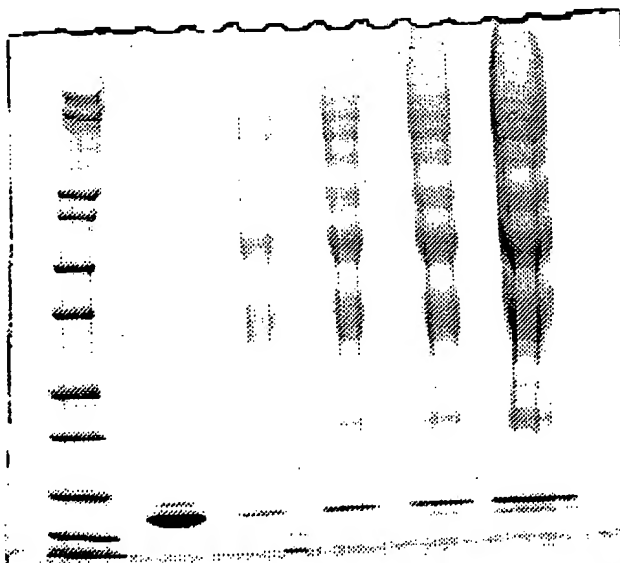
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	50% of Conc (mg/ml)	Load uL	Load ug
MW STD	1			15	
	2				
GCSF 7559-11	3	1.00	0.50	10.0	5.00
	4				
TRI-TET. 102193-1	5	2.00	1.00	5.0	5.00
	6				
TRI-TET. 102193-1	7	2.00	1.00	10.0	10.00
	8				
TRI-TET. 102193-1	9	2.00	1.00	15.0	15.00
	10				
TRI-TET. 102193-1	11	2.00	1.00	20.0	20.00
	12				



To Page No. 1

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Turner

Recorded by

Date

Project No. 102003Book No. 5575

TITLE

Various Amounts of Tri-Tetra PEG-GCSF Eluted off Affinity ColumnFrom Page No. X

PEG-GCSF-

REDACTED

Date:

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

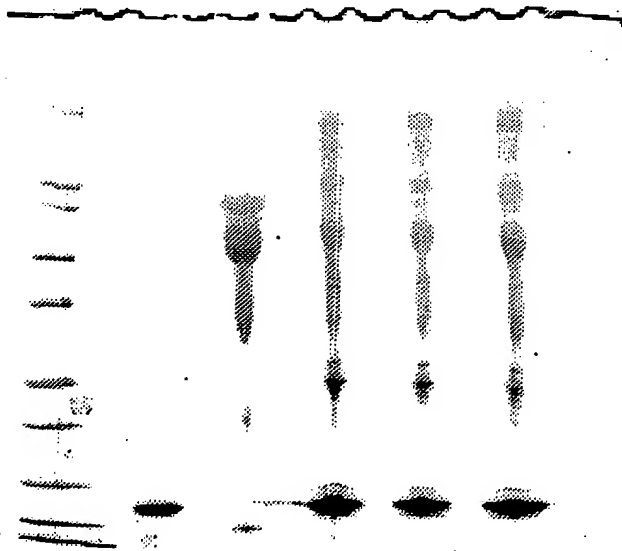
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW STD	1			15	
	2				
GCSF #7559-11	3	1.00	0.75	6.7	5.00
	4				
TRI-TET. #7559-06	5	1.00	0.75	13.3	10.00
	6				
TRI-TET. 102293-1	7	1.38	1.04	19.3	20.00
	8				
TRI-TET. 102393-1	9	0.90	0.68	29.6	20.00
	10				
TRI-TET. 102493-1	11	0.90	0.68	29.6	20.00
	12				

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Initiated by

Christine Jansen

Date

Recorded by

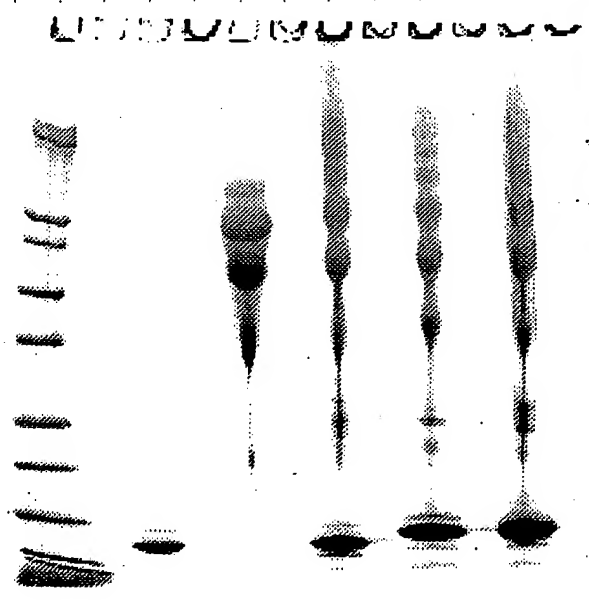
From Page No. 2

PEG-GCSF-

REDACTED

Date: _____
Operator: Chris
PEG-GCSF _____
NB No: _____
4-20% Gradient Mini Gel _____
Running Conditions
constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW STD	1			15	
	2				
GCSF #7559-11	3	1.00	0.75	6.7	5.00
	4				
TRI-TET. #7559-06	5	1.00	0.75	13.3	10.00
	6				
TRI-TET. NON-REDUCED	7	1.38	1.04	19.3	20.00
	8				
TRI-TET. REDUCED-DTT	9	0.90	0.68	29.6	20.00
	10				
TRI-TET. REDUCED-ABDF	11	0.90	0.68	29.6	20.00
	12				



To Page No. X

Witnessed & Understood by me, <u>William Allerton</u>	Date	Invented by <u>Christine Farrah</u>	Date
		Recorded by	

Project No. 102003Book No. 5575TITLE Reduced N-Term PEG-GCSF Eluted off Affinity ColumnFrom Page No. X

PEG-GCSF-

REDACTED

Date: _____

Operator: Chris

PEG-GCSF

NB No: _____

4-20% Gradient Mini Gel

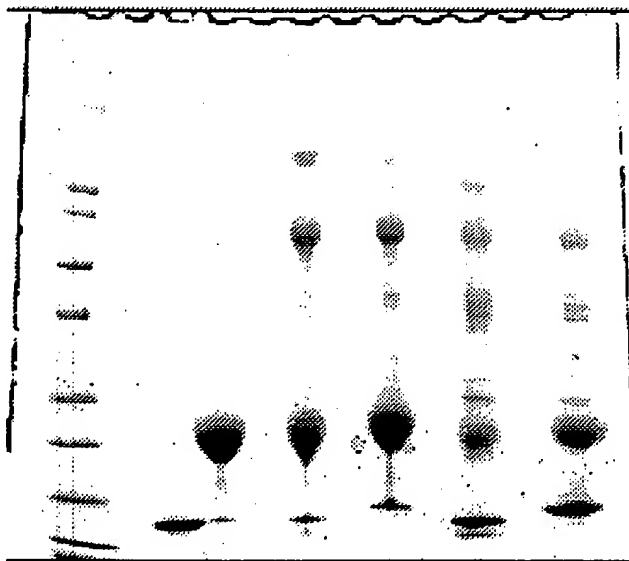
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75-66.6% Conc(mg/ml)	Load uL	Load ug
MW STD	1			15	
	2				
GCSF #7559-11	3	1.00	0.75	6.7	5.00
N-TERM #7559-01	4	1.00	0.75	13.3	10.00
	5				
N-TERM NON-REDUCED	6	1.00	0.75	20.0	15.00
	7				
N-TERM REDUCED	8	1.00	0.67	22.5	15.00
	9				
N-TERM + GCSF NON-REDUCED	10	1.00	0.75	20.0	15.00
	11				
N-TERM + GCSF REDUCED	12	1.00	0.67	22.5	15.00

To Page No. X

Witnessed & Understood by me,

William Callahan

Date

Invented by

Christine Farrar

Date

Recorded by

From Page No. X

REDACTED

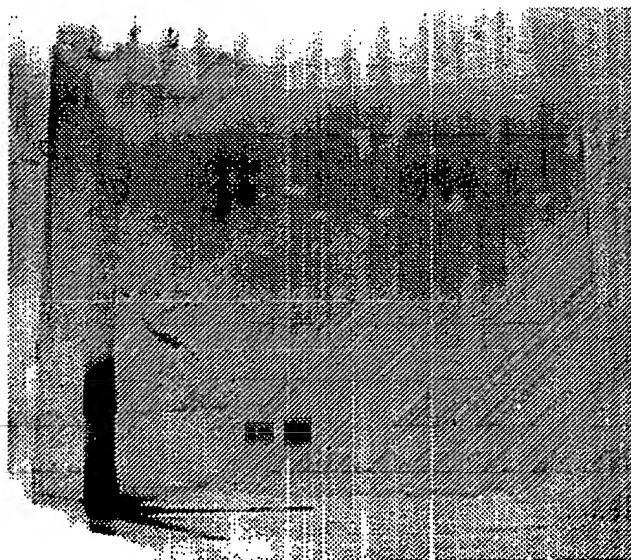
AP WESTERN BLOT

Date: _____
Operator: Chris
Alkaline Phosphatase Reagent

NB No: _____
4-20% Gradient Mini Gel

Running Conditions constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/AP Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load ng	Load uL
GCSF Std T6702	1	1.00	200	5.00	3.75	25	7
	2						
N-TERM #7559-01	3	1.00	4000	0.25	0.19	1	5
N-TERM #7559-01	4	1.00	1000	1.00	0.75	5	7
N-TERM #7559-01	5	1.00	1000	1.00	0.75	10	13
N-TERM #7559-01	6	1.00	200	5.00	3.75	25	7
N-TERM #7559-01	7	1.00	200	5.00	3.75	50	13
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	5	7
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	10	13
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	25	7
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	50	13
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	100	13



To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Christine Farnsworth

Recorded by

Project No. 102003Book No. 5575TITLE Horse Radish Peroxidase (Biotinylated) Trial Western BlotFrom Page No. X

REDACTED

HRP WESTERN BLOT

Date: _____

NB No: _____

Operator: Chris

4-20% Gradient Mini Gel

Horse Radish Peroxidase Reagent

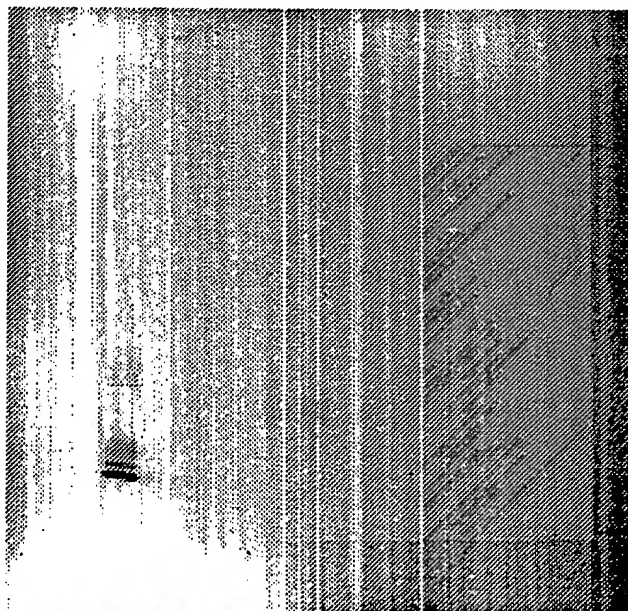
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/HRP Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load ng	Load uL
GCSF Std T6702	1	1.00	200	5.00	3.75	25	7
	2						
N-TERM #7559-01	3	1.00	4000	0.25	0.19	1	5
N-TERM #7559-01	4	1.00	1000	1.00	0.75	5	7
N-TERM #7559-01	5	1.00	1000	1.00	0.75	10	13
N-TERM #7559-01	6	1.00	200	5.00	3.75	25	7
N-TERM #7559-01	7	1.00	200	5.00	3.75	50	13
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	5	7
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	10	13
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	25	7
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	50	13
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	100	13

To Page No. X

Witnessed & Understood by me,

Vallian Allston

Date

Invented by

Christina E. Farrar

Recorded by

Date

111

TITLE Alkaline Phosphatase Trial Western Blot (2)

Project No. 102003

Book No. 5575

81

From Page No. X

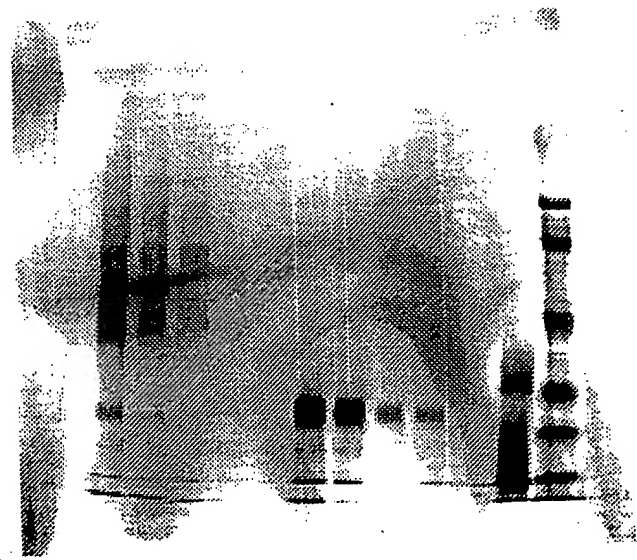
AP WESTERN BLOT

REDACTED

Date: _____ NB No: _____
Operator: Chris 4-20% Gradient Mini Gel
Alkaline Phosphatase Reagent

Running Conditions constant current: 25 mA
all non-reduced
4-20% Gradient Mini Gel/AP Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load uL	Load ng
PRESTAINED STDS	1					10	
GCSF Std T6702	2	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	3	1.00	4000	0.25	0.19	5	1
N-TERM #7559-01	4	1.00	1000	1.00	0.75	7	5
N-TERM #7559-01	5	1.00	1000	1.00	0.75	13	10
N-TERM #7559-01	6	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	7	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	7	5
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	13	10
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	7	25
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	13	100



To Page No. X

Witnessed & Understood by me,
Kellin Callahan

Date

Invented by Christine Jones
Recorded by _____

Date

Project No. 10003Book No. 5575TITLE Horse Radish Peroxidase Trial Western Blot (2)From Page No. X

HRP WESTERN BLOT

REDACTED

Date: _____

NB No: _____

Operator: Chris

4-20% Gradient Mini Gel

Horse Radish Peroxidase Reagent

Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/HRP Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load uL	Load ng
PRESTAINED STDS	1					10	
GCSF Std T6702	2	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	3	1.00	4000	0.25	0.19	5	1
N-TERM #7559-01	4	1.00	1000	1.00	0.75	7	5
N-TERM #7559-01	5	1.00	1000	1.00	0.75	13	10
N-TERM #7559-01	6	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	7	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	7	5
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	13	10
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	7	25
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	13	100

To Page No. X

Witnessed & Understood by me,

Date

Invested by

Date

5023-10-01

Recorded by

indd 11

From Page No. X

ECL WESTERN BLOT

REDACTED

Date: _____

NB No: _____

Operator: Chris

4-20% Gradient Mini Gel

Enhanced chemiluminescence

Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/ECL Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load uL	Load ng
PRESTAINED STDS	1					10	
GCSF Std T6702	2	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	3	1.00	4000	0.25	0.19	5	1
N-TERM #7559-01	4	1.00	1000	1.00	0.75	7	5
N-TERM #7559-01	5	1.00	1000	1.00	0.75	13	10
N-TERM #7559-01	6	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	7	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	7	5
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	13	10
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	7	25
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	13	100

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William CallahanChristine Farrar

Recorded by

Project No. 10223Book No. 5575TITLE Enhanced Chemiluminescence Trial Western Blot-DuPontFrom Page No. X

ECL WESTERN BLOT-DuPont

REDACTED

Date: _____

NB No: _____

Operator: Chris

4-20% Gradient Mini Gel

Enhanced chemiluminescence

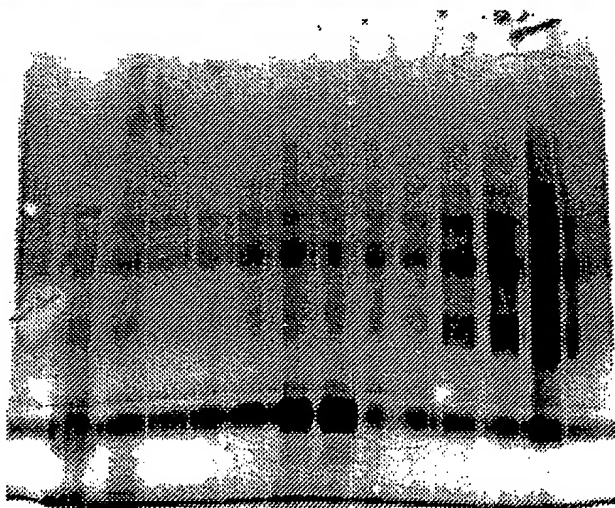
Running Conditions

constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/ECL Western Blot Procedure

Sample Code	Lane No.	Conc (mg/ml)	Dilution Factor	Dil. Conc (ng/ul)	75% Conc (ng/ul)	Load uL	Load ng
PRESTAINED STDS	1					10	
GCSF Std T6702	2	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	3	1.00	4000	0.25	0.19	5	1
N-TERM #7559-01	4	1.00	1000	1.00	0.75	7	5
N-TERM #7559-01	5	1.00	1000	1.00	0.75	13	10
N-TERM #7559-01	6	1.00	200	5.00	3.75	7	25
N-TERM #7559-01	7	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	8	1.00	1000	1.00	0.75	7	5
TRI-TETRA #7559-06	9	1.00	1000	1.00	0.75	13	10
TRI-TETRA #7559-06	10	1.00	200	5.00	3.75	7	25
TRI-TETRA #7559-06	11	1.00	200	5.00	3.75	13	50
TRI-TETRA #7559-06	12	1.00	100	10.00	7.50	13	100

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

William AllenChris Farnon

From Page No. X

PEG-GCSF WEST (1)

REDACTED

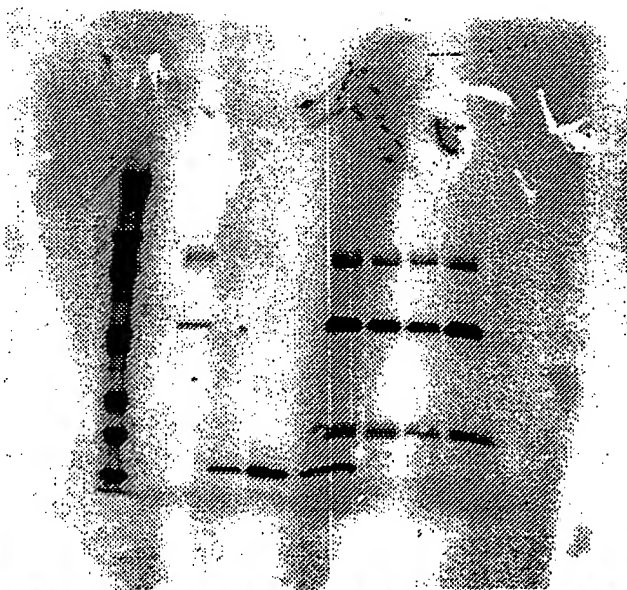
Date: _____
Operator: Chris Farrar
GCSF from hamster study G083193
purified sera samples/not concentrated

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all reduced/BME
Western Blot SOP (DuPont ECL development)

Sample Code	LANE	est. conc. (ng/ml)	75% of conc. ng/ml	ng	ul
PRE STAINED MARKER	1				10.00
	2				
VEHICLE (T=4, G1)	3	100	75	0.80	10.67
GCSF #7559-11	4	100	75	0.80	10.67
GCSF RUN OVER COLUMNS	5	100	75	0.80	10.67
	6				
T=.5	7	40	30	0.80	26.67
T=1	8	40	30	0.80	26.67
T=1.5	9	40	30	0.80	26.67
T=2	10	40	30	0.80	26.67
	11				
	12				



To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Collins

Christine Farrar

Recorded by

Project No. 18003
Book No. 5575TITLE Dilute Sera Samples Purified Group N from GFrom Page No. 2

PEG-GCSF WEST (2)

REDACTED

Date: _____

Operator: Chris Farrar

NB No: _____

N-Term PEG-GCSF from study G083193
purified sera samples/not concentrated

4-20% Gradient Mini Gel

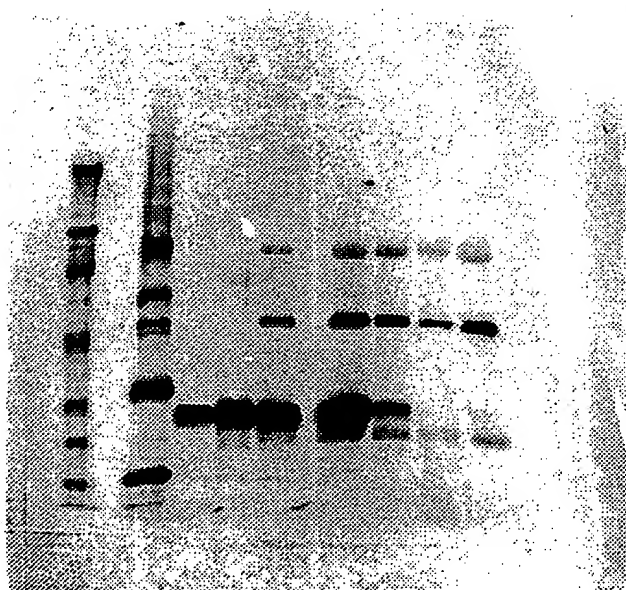
Running Conditions

constant current: 25 mA

all reduced/BME

Western Blot SOP (DuPont ECL development)

Sample Code	LANE	est. conc. (ng/ml)	75% of conc. ng/ml	ng	ul
PRE STAINED MARKER	1				10.00
	2				
VEHICLE (T=2, G1)	3	100	75	0.80	10.67
N-TERM PEG-GCSF #7559-01	4	100	75	0.80	10.67
N-TERM RUN OVER COLUMN	5	100	75	0.80	10.67
N-TERM SPIKED SERA	6	100	75	0.80	10.67
	7				
T=.5	8	40	30	0.80	26.67
T=1	9	40	30	0.80	26.67
T=1.5	10	40	30	0.80	26.67
T=2	11	40	30	0.80	26.67
	12				

To Page No. 2

Witnessed & Understood by me,

Date

Invented by

Date

William Allalou

Recorded by

Christine Farrar

From Page No. 7

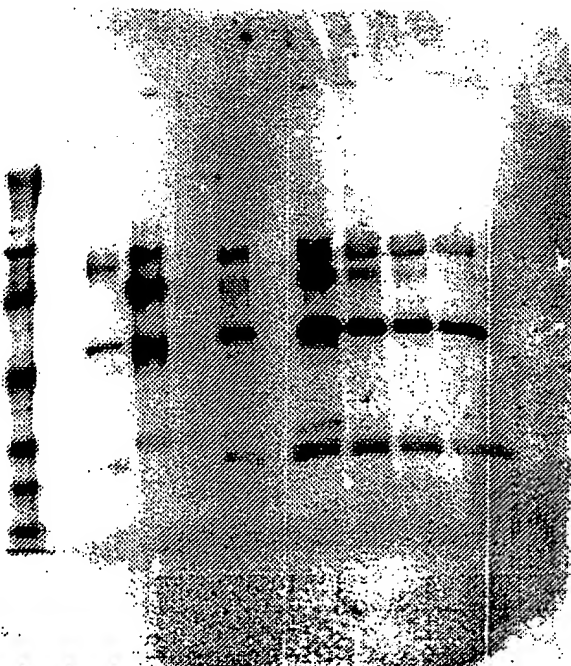
REDACTED

PEG-GCSF WEST (3)

Date: _____
Operator: Chris Farrar NB No: _____
Tri/tetra PEG-GCSF from study G083193 4-20% Gradient Mini Gel
purified sera samples/not concentrated

Running Conditions
constant current: 25 mA
all reduced/BME
Western Blot SOP (DuPont ECL development)

Sample Code	LANE	est. conc. (ng/ml)	75% of conc. ng/ml	ng	ul
PRE STAINED MARKER	1				10.00
	2				
VEHICLE (T=7, G1)	3	100	75	0.80	10.67
TRI/TETRA PEG-GCSF #7559-06	4	100	75	0.80	10.67
TRI/TETRA RUN OVER COLUMNS	5	100	75	0.80	10.67
TRI/TETRA SPIKED SERA	6	100	75	0.80	10.67
	7				
T=.5	8	40	30	0.80	26.67
T=1	9	40	30	0.80	26.67
T=1.5	10	40	30	0.80	26.67
T=2	11	40	30	0.80	26.67
	12				



To Page No. 8

Witnessed & Understood by me,
Kellin Callahan

Date _____

Invented by Chris Farrar
Recorded by _____

Date _____

Project No. 10003
Book No. 5515

TITLE MW Markers and Serum Proteins from Blank Sera

From Page No. 7

PEG-GCSF

REDACTED

Date:

Operator: Chris

MW Markers and Serum Proteins

NB No:

4-20% Gradient Mini Gel

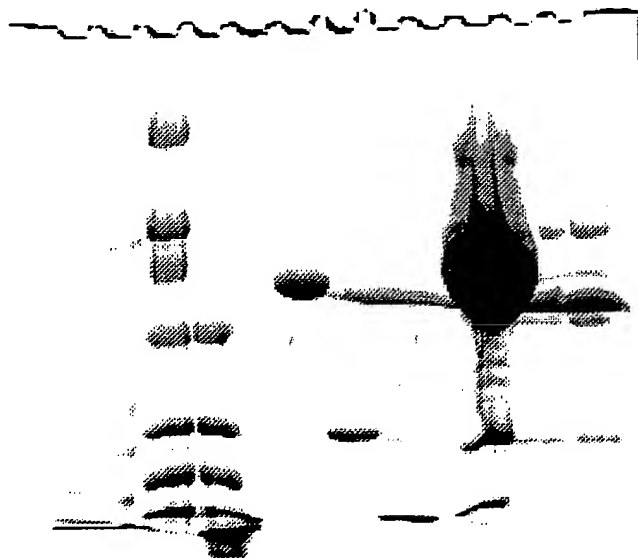
Running Conditions

constant current: 25 mA

all reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std NOVEX	1	1.00		15	15
MW std BIORAD	2	1.00		10	10
HI MW std AMERSHAM	3	1.40	1.05	14	15
LO MW std AMERSHAM	4	1.40	1.05	14	15
	5				
ALBUMIN (66,000)	6	2.00	1.50	7	10
CARBONIC ANHYDRASE (29,000)	7	2.00	1.50	7	10
CYTOCHROME C (12,400)	8	2.00	1.50	7	10
	9				
hamster serum	10	1.00	0.75	13	10
hamster immunoglobulins	11	0.50	0.38	13	5
hamster immunoglobulins	12	0.50	0.38	27	10



To Page No. 7

Witnessed & Understood by me,

Villiam Ollala

Date

Invented by

Christine Janson

Date

Recorded by

From Page No. X

REDACTED

PEG-GCSF

Date:

Operator: Chris
Hamster Serum Proteins

NB No:

4-20% Gradient Mini Gel

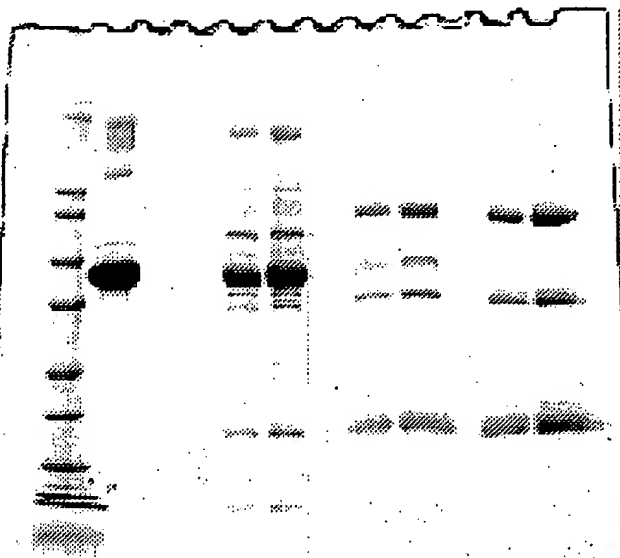
Running Conditions

constant current: 25 mA

all reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std NOVEX	1	1.00		15	15
ALBUMIN (66,000)	2	2.00	1.50	7	10
	3				
	4				
diluted hamster serum	5	5.00	3.75	1	5
diluted hamster serum	6	5.00	3.75	3	10
	7				
IgG (protein A col. eluent)	8	0.66	0.50	10	5
IgG (protein A col. eluent)	9	0.66	0.50	20	10
	10				
sample (protein A col. wash)	11	0.66	0.50	10	5
sample (protein A col. wash)	12	0.66	0.50	20	10



To Page No. X

Witnessed & Understood by me,

Vallini Cellabim

Date

Invented by

Christine Gosses
Recorded by

Date

Project No. 1003Book No. 557D

TITLE

Protein A column Wash & Eluent from Blank Sep Non-Red.From Page No. X

REDACTED

PEG-GCSF

Date:

Operator: Chris

Hamster Serum Proteins

NB No:

4-20% Gradient Mini Gel

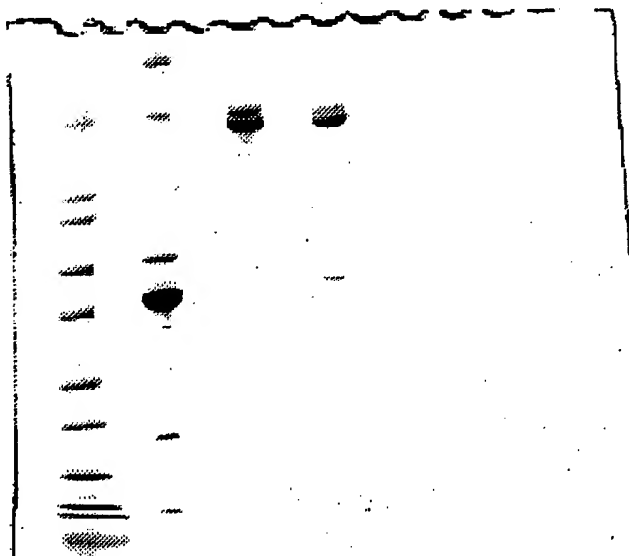
Running Conditions

constant current: 25 mA

all NON-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std NOVEX	1	1.00		15	15
	2				
diluted hamster serum	3	5.00	3.75	3	10
	4				
sample (protein A col. wash)	5	0.66	0.50	20	10
	6				
IgG (protein A col. eluent)	7	0.66	0.50	20	10
	8				
	9				
	10				
	11				
	12				

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Recorded by

Christine Faison

From Page No. X

REDACTED

Date:

Operator: Chris

Hamster Serum Proteins

NB No:

4-20% Gradient Mini Gel

Running Conditions

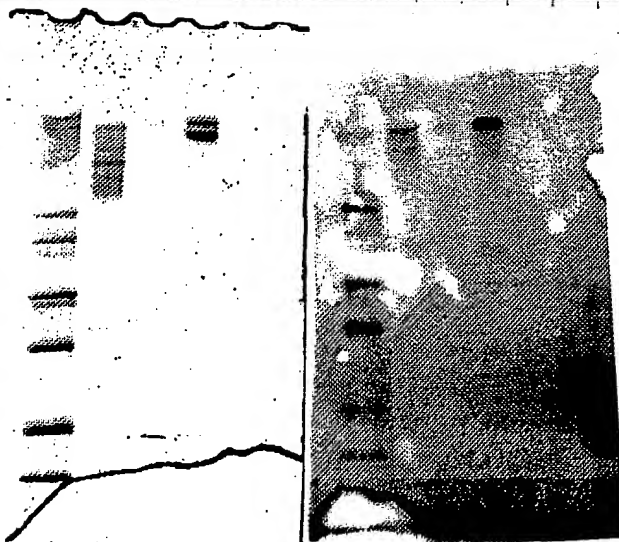
constant current: 25 mA

all NON-reduced

4-20% Gradient Mini Gel

1/2 comasie SOP: 1/2 Western ECL

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std NOVEX	1				
wash from 1st protein A col.	2	1.00	1.00	15	15.00
wash from 2nd protein A col.	3	0.15	0.11	26	2.93
eluent from 2nd protein A col.	4	0.01	0.01	26	0.17
	5	0.12	0.09	26	2.26
	6				
	7				
MW std NOVEX	8	1.00	1.00	15	15.00
wash from 1st protein A col.	9	0.15	0.11	26	2.93
wash from 2nd protein A col.	10	0.01	0.01	26	0.17
eluent from 2nd protein A col.	11	0.12	0.09	26	2.26
	12				

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William CallahanChristine Turner

Recorded by

Project No. 10803Book No. 5575

TITLE

Purified GCSF sera samples / concentrated and reducedFrom Page No. X

PEG-GCSF WEST (1)

REDACTED

Date:

Operator: Chris Farrar

NB No:

GCSF from hamster study G083193
purified sera samples/concentrated

4-20% Gradient Mini Gel

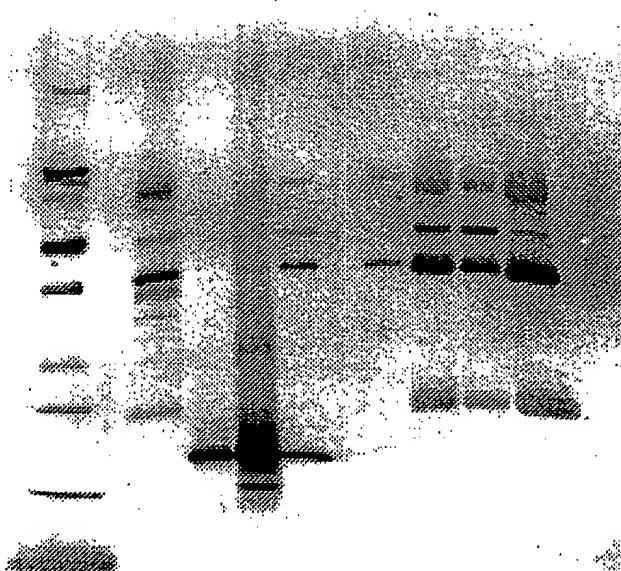
Running Conditions

constant current: 25 mA

all reduced/BME

Western Blot SOP (DuPont ECL development)

Sample Code	LANE	est. conc. (ng/ml)	75% of conc. ng/ml	ng	ul
PRE STAINED MARKER	1				10
	2				
VEHICLE (T=4, G1)	3				27
GCSF #7559-11	4	100	75	0.80	11
GCSF RUN OVER COLUMNS	5	100	75	0.80	11
GCSF SPIKED SERUM	6	100	75	0.80	11
	7				
T=5	8	40	30	0.80	27
T=1	9	40	30	0.80	27
T=1.5	10	40	30	0.80	27
T=2	11	40	30	0.80	27
	12				

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Allen

Recorded by

Christine Farrar

From Page No. 4

PEG-GCSF WEST (2)

REDACTED

Date:

Operator: Chris Farrar

GCSF from hamster study G083193

purified sera samples/concentrated

NB No:

4-20% Gradient Mini Gel

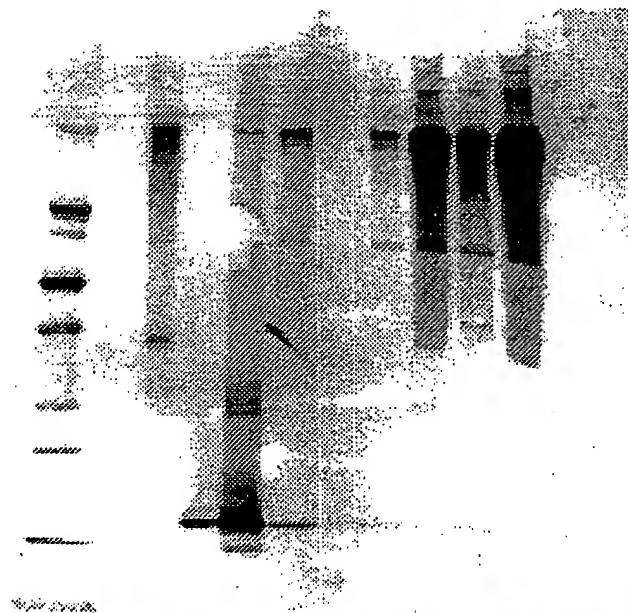
Running Conditions

constant current: 25 mA

all non reduced/BME

Western Blot SOP (DuPont ECL development)

Sample Code	LANE	est. conc. (ng/ml)	75% of conc. ng/ml	ng	ul
PRE STAINED MARKER	1				10
	2				
VEHICLE (T=4, G1)	3				27
GCSF #7559-11	4	100	75	0.80	11
GCSF RUN OVER COLUMNS	5	100	75	0.80	11
GCSF SPIKED SERUM	6	100	75	0.80	11
	7				
T=5	8	40	30	0.80	27
T=1	9	40	30	0.80	27
T=1.5	10	40	30	0.80	27
T=2	11	40	30	0.80	27
	12				



To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Recorded by

Christine Farrar

Project No. 10003Book No. 5570TITLE GCSF FPLC Protein A fractions fromFrom Page No. X

REDACTED

PEG-GCSF

Date: _____

Operator: Chris

FPLC Protein A column fractions

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

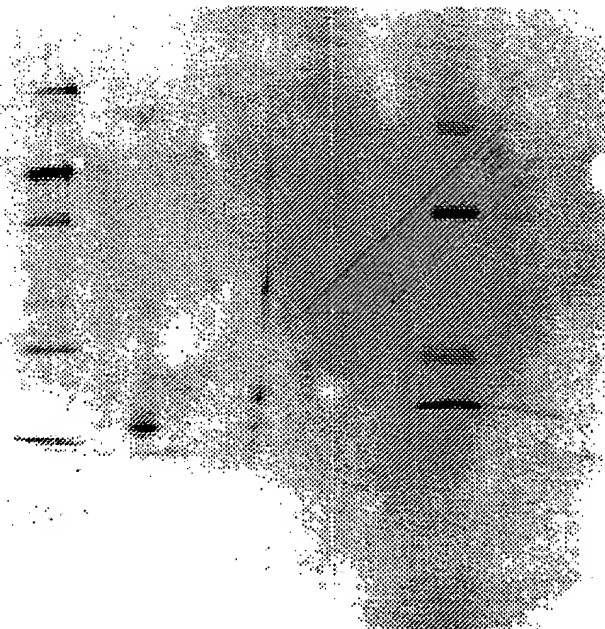
constant current: 25 mA

all reduced

4-20% Gradient Mini Gel

Western Blot SOP (DuPont ECL development)

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load ng	Load uL
MW std NOVEX	1				15
	2				
GCSF #7559-11	3	100.00	75.00	1.00	13
	4				
040494-1 FXN 3	5	40.00	30.00	0.90	30
040494-1 FXN 4	6	40.00	30.00	0.90	30
040494-1 FXN 5	7	40.00	30.00	0.90	30
040494-1 FXN 6	8	40.00	30.00	0.90	30
040494-1 FXN 7	9	40.00	30.00	0.90	30
	10				
	11				
	12				

To Page No. X

Witnessed & Understood by me,

William Allen

Date

Invented by

Christine Jones

Date

Recorded by

From Page No. 7

REDACTED

PEG-GCSF

Date:

Operator: Chris

FPLC Protein A column fractions

NB No:

4-20% Gradient Mini Gel

Running Conditions

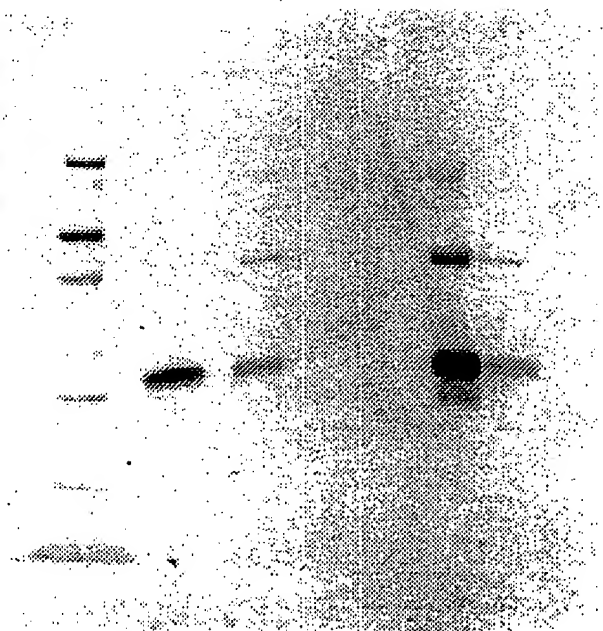
constant current: 25 mA

all reduced

4-20% Gradient Mini Gel

Western Blot SOP (DuPont ECL development)

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load ng	Load uL
MW std NOVEX	1				15
	2				
N-TERM #7559-01	3	100.00	75.00	1.00	13
	4				
040494-2 FXN 1+2	5	40.00	30.00	0.90	30
040494-2 FXN 3	6	40.00	30.00	0.90	30
040494-2 FXN 4	7	40.00	30.00	0.90	30
040494-2 FXN 5	8	40.00	30.00	0.90	30
040494-2 FXN 6	9	40.00	30.00	0.90	30
040494-2 FXN 7	10	40.00	30.00	0.90	30
	11				
	12				



To Page No. 8

Witnessed & Understood by me,

Date

Invented by

Date

William Callahan

Recorded by

Christine Farnsworth

Project No. 10043Book No. 5575TITLE TRI-TETRA FPLC Protein A fractions fromFrom Page No. 8

REDACTED

PEG-GCSF WEST

Date: _____

Operator: Chris

FPLC Protein A column fractions

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

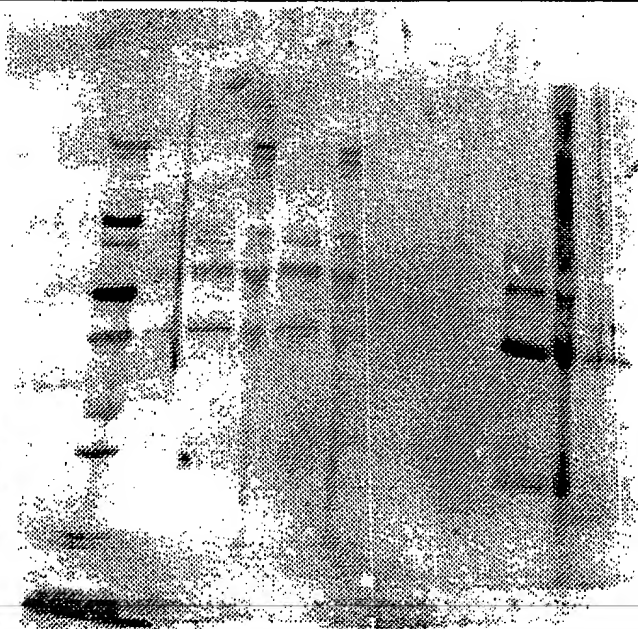
constant current: 25 mA

all reduced

4-20% Gradient Mini Gel

Western Blot SOP (DuPont ECL development)

Sample Code	Lane No.	Conc (ng/ml)	75% of Conc (ng/ml)	Load ng	Load uL
MW std NOVEX	1				15
TRI-TETRA #7559-06	2	100.00	75.00	1.00	13
040494-3 FXN 1	3	40.00	30.00	0.90	30
040494-3 FXN 1 (non-reduced)	4	40.00	30.00	0.90	30
040494-3 FXN 2	5	40.00	30.00	0.90	30
040494-3 FXN 2 (non-reduced)	6	40.00	30.00	0.90	30
040494-3 FXN 3	7	40.00	30.00	0.90	30
040494-3 FXN 4	8	40.00	30.00	0.90	30
040494-3 FXN 5	9	40.00	30.00	0.90	30
040494-3 FXN 6	10	40.00	30.00	0.90	30
040494-3 FXN 6 (non-reduced)	11	40.00	30.00	0.90	30
040494-3 FXN 7	12	40.00	30.00	0.90	30

To Page No. 8

Witnessed & Understood by me,

Date

Invented by

Date

William Collier

Recorded by

Christine Farnan5/15/01

LABORATORY
NOTEBOOK

No 5576

AMGEN

REDACTED

Box 0608

REDACTED

NOTEBOOK NO. 5576
ISSUED TO Christine Farrar
ON _____ 19____
DEPARTMENT 215
RETURNED _____ 19____

"MICROFILMED"

DATE _____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

INSTRUCTIONS

1. The primary purpose of this notebook is to protect your and the Company's Patent-Rights by keeping records of all original work in a form acceptable as evidence if any legal conflict arises.
2. When starting a page, enter the title, project number, and book number. Use ink for permanence—no pencil. Record your work as you progress, including any spur-of-the-moment ideas which may be developed later. Do not make notes on loose paper to be recopied. Use the blank lefthand page for calculations so they will be available if you want to re-check them. Record your work in such a manner that a co-worker can continue from where you stop. You might be ill and to protect your priority it could be urgent that the work continue while you are absent.
3. Give a complete account of your experiments and the results, both positive and negative, including your observations. Record all diagrams, layouts, plans, procedures, new ideas, or anything pertinent to your work including the details of any discussions with suppliers, or other people outside the Company. Do not try to erase any incorrect entries; draw lines deleting them, note the corrections, sign and date the changes. This extra care is worthwhile because of the necessity of original data to prove priority of new discoveries.
4. After entering your data, sign and date the entries. Explain your work to at least two witnesses who are not co-inventors, and have them sign and date the pages in the place provided. Record the names of operators and witnesses present during any demonstration and have at least two witnesses sign the page. If no witnesses are present during an experiment of importance, repeat it in the presence of two witnesses.
5. This notebook and its contents are the exclusive property of the Company. It is confidential and the contents are not to be disclosed to anyone unless authorized by the Company. You must return it when completed, upon request, or upon termination of employment. It should be kept in a protected place. If loss occurs notify your supervisor immediately, and make a written report describing the circumstances of the loss.

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TLE Vidling PEG-rhu-GCSF lot No. 4656-47

Project No. 102003

Book No. 5576

1

From Page No. X

PEG-rhu-GCSF 1.0mg/ml
Lot No. 4656-47

Bulk protein obtained from Randy DeRince at 1.0mg/ml
Final steril filtration done in laminar flow hood w/ 1 l Nalgene CA 0.2 μ filter unit
2 ml dispensed into 3 ml vials & sealed w/ 13 mm seals

368 - 2ml Vials stored c 4c

Radiometer microprobe

- 1 pH of pH 4.0 standard at room temp. - 4.01
- 2 pH of 1/2 ml sample in 3 ml vial at 4°C - 4.04
- 3 pH of (2) in epindorph at 4°C - 4.02
- 4 pH of 1/2 ml sample returned to 3 ml vial - 4.01

REDACTED

To Page No. X

Witnessed & Understood by me,

Bar Oeis

Date

Invented by

Christine Farnar

Date

Recorded by

Project No. 102003Book No. 5576TITLE Study- Shipment to Nobel Researchom Page No. X

REDACTED

Materials: GCSE lot #683, 2.6 mg/ml Pure bulk, 50140, in 10mM NaOAc,
5% Mannitol, .004% Tween 80 pH 3.25

PEG-GCSE lot #4656-47, ~~2.6~~ 1.0 mg/ml in 10mM NaOAc,
5% Mannitol, .004% Tween 80 pH 4.0

Standard GCSE buffer: 10mM NaOAc, 5% Mannitol, .004% Tween 80
pH 4.0

PLA-PEG 2:1 PLA(Mn-1,000)-PEG(Mn 1,000) copolymer solution
in water (30% w/w), lot no. 4987-69.

5cc Sterile Pyrogen Free Sample Vials ~~X4~~ X6 X4

13 mm West rubber seals ~~X4~~ X6 X4

13 mm flip top crimp seals ~~X4~~ X6 X4

Gelman filter, .2 um sterile Acrodisc, product no. 4192 ~~X4~~ X6 X4

To Page No. 3

Witnessed & Understood by me,

R. E. Lee

Date

Invented by

Date

Recorded by Christine Farrar

REDACTED

From Page No. 2

Procedure: Group 1 - filtered 7ml of Standard GCSF buffer into steril 15ml centrifuge tube. Pipetted 5.0ml of steril buffer into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Group 2 - Determined protein concentration of GCSF lot #683 by ~~8280~~ A_{280}
 $2.20399 A_{280} / .86 A_{280} \text{ ml/mg} = 2.563 \text{ mg/ml}$
 measured volume .770ml

$$.77 \text{ ml} \times 2.563 \text{ mg/ml} = X \times 1.0 \text{ mg/ml}$$

$$X = 1.9735 \text{ ml}$$

$$1.9735 \text{ ml} - .77 \text{ ml} = 1.204 \text{ ml}$$

Added 1.204 ml of Standard Buffer to 0.77 ml of GCSF solution to give a 1mg/ml solution. Protein concentration was checked by A_{280}

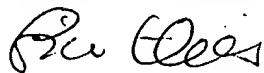
Peaks : L224=2.28545 L280=0.85550 L582=0.01128 L486=0.00978
 (Stdev) : (0.095) (0.0011) (0.00019) (0.00012)

$$.8555 A_{280} / .86 A_{280} \text{ ml/mg} = 1.0 \text{ mg/ml}$$

Took 800ul of 1.0mg/ml GCSF solution and diluted 10X with 7.2 ml of Standard G Buffer to give a 0.1mg/ml solution. Filtered 7ml of this solution into steril 15ml centrifuge tube. Pipetted 5.0ml of steril buffer into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

To Page No. 4

Witnessed & Understood by me,



Date

Invented by

Date

Recorded by

Christine Farnon

From Page No. 3

Group 3 - Remaining 1.0 mg/ml GCSF solution was steril filtered into steril epidural tube. Pippeted .556 ml of steril 1.0 mg/ml GCSF solution into 10 ml vial containing 5.0 ml of PLA-PEG copolymer solution lot # 498769. Mixed by inverting vigorously for 2 min. Pippeted this solution (5.0 ml) into 5 ml injection vial under aseptic conditions. Sealed vial with 13mm rubber seal and 13mm flip top crimp seal.

Group 4 - Pippeted 0.556 ml of steril 1.0 mg/ml GCSF solution PEG-GCSF lot # 4656-47 into 10 ml vial containing 5.0 ml of PLA-PEG copolymer solution lot # 498769. Mixed by inverting vigorously for 2 min. Pippeted 5 ml of this solution into 5 ml injection vial under aseptic conditions. Sealed vial with 13mm rubber seal and 13mm flip top crimp seal.

Vials were labeled and packaged on wet ice and shipped to Nobel Research on 5/1/95. Procedure done same day as shipment.

To Page No. 45

Witnessed & Understood by me,

for Elie

Date

Invented by

Date

Recorded by

Christine Farnar

TITLE Study

Project No. 102003

Book No. 5576

5

From Page No. 4

REDACTED

CONFIDENTIAL

Filename:

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Study No.

Treatment	Vehicle	Lot No.	Daily Dose (mg/kg)	Group	Animals per Group	Inject. Volume (mL)	Average Weight (kg)	Dosing Conc. (mg/mL)	Nominal Dosing Solution (mL)	Actual Dosing Solution (mL)	Number of Vials	G-CSF (mg)	Total G-CSF (mg)
Vehicle	Aq. Form.	NA	NA	1	28	0.1	0.1	NA	2.8	5.0	1	NA	NA
rhu-G-CSF	Aq. Form.	683	0.1	2	35	0.1	0.1	0.10	3.5	5.0	1	0.500	
rhu-G-CSF	PLA-PEG	683	0.1	3	35	0.1	0.1	0.10	3.5	5.0	1	0.500	
PEG-rhu-G-CSF	PLA-PEG	4656-47	0.1	4	35	0.1	0.1	0.10	3.5	5.0	1	0.500	1.500
Totals =					133				13.3	20	4		

Animals: Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule: Single s.c. dose on day 1 (M)

Sacrifice Schedule: Four animals from group 1 will be bled and sacrificed at intervals of 0.5, 1, 1.5, 2, 4, 7 and 10 days following dosing.

Five animals from each of groups 2-4 will be bled and sacrificed at intervals of 0.5, 1, 1.5, 2, 4, 7 and 10 days following dosing.

Injection Notes: Use one vial for each treatment group
Fill a 1-mL syringe, and inject up to ten hamsters (0.1 mL per animal)
No need to change syringe needles between hamsters within a single group
Vials are overfilled by 1.5 mL to allow for losses in filling syringes

Vehicles:

Aqueous formulation = 10 mM NaOAc, 5 % (w/v) mannitol, 0.004 % (w/v) Tween 80, pH 4.0

PLA-PEG = 2:1 PLA(Mn 1,000)-PEG(Mn 1,000) copolymer solution in water (30 % w/w), lot no. 4987-69

Dosing solutions prepared by adding 0.556 mL of 1.0 mg/mL rhu-G-CSF or PEG-rhu-G-CSF in aqueous formulation to 5.0 mL PLA-PEG

Volume per vial: 5.0 mL

To Page No. X

Witnessed & Understood by me,

[Signature]

Date

Invented by

Date

Recorded by

[Signature]

Project No. 102603Book No. 5576TITLE StudyShipment to Nobel ResearchFrom Page No. X

Materials: Standard GCSF buffer: 10mM NaOAc, 5% Mannitol, .004% Tween 80
pH 4.0

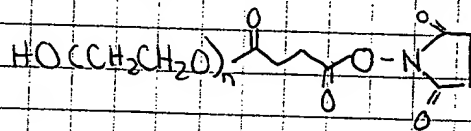
GCSF lot 636J0A, ^{0.3} ~~0.6~~ mg/ml in Standard GCSF buffer pH 4.0

WFI, water for irrigation, Kendall McGaw

500mM Bicine pH 8.0

SS-PEG, (50K) lot #PD-498-KIC

REDACTED



Mono-PEGYLATED GCSF (4656-71) from Notebook 4656 page 71

DI-PEGYLATED GCSF (4656-72) from Notebook 4656 page 72

5cc Sterile Pyrogen Free Sample Vials #4x6

13 mm West rubber seals #4x6

13 mm Flip top crimp seals #4x6

Gelman filter, .2um sterile Acrodisc, product no. 4192 #4x6

Witnessed & Understood by me,

Date

Invented by

Date

To Page No. 7

Recorded by

111

TITLE

Study

Project No. 102003

Book No. 5576

7

REDACTED

From Page No. 6

Procedure: Group 1 - filtered 5ml of Standard GCSF buffer into 5ml injection vial under aseptic conditions. Sealed vial w/ 13 mm rubber seal and 13mm flip top crimp seal.

Group 2 - added 4.00 ml of Standard GCSF buffer to 2.00 ml of GCSF lot 636J0A (13^{mg}/ml) to give a .1^{mg}/ml solution. Filtered 5ml of this solution into 5ml injection vial under aseptic conditions. Sealed vial w/ 13 mm rubber seal and 13mm flip top crimp seal.

Group 3 - filtered 5ml of .1^{mg}/ml Mono-PEG GCSF from Notebook 4656-71 into 5ml injection vial under aseptic conditions. Sealed vial w/ 13 mm rubber seal and 13mm flip top crimp seal. There was enough Mono PEG GCSF to filter another 5ml into another injection vial under aseptic conditions. The extra vial was sealed w/ 13 mm rubber seal and 13 mm flip top crimp seal and stored at 4°C.

Group 4 - Filtered 5ml of .1^{mg}/ml Di-PEG GCSF from Notebook 4656-72 into 5ml injection vial under aseptic conditions. Sealed vial w/ 13 mm rubber seal and 13mm flip top crimp seal. There was enough Di-PEG GCSF to filter another 5ml into another injection vial under aseptic conditions. The extra vial was sealed w/ 13 mm rubber seal and 13mm flip top crimp seal and stored at 4°C.

Vials were labeled and packaged on wet ice and shipped to Nobel Research on . Procedure done same day as shipment.

To Page No. 8

Witnessed & Understood by me,

Ben Oels

Date

Invented by

Date

Recorded by

Christine Turner

Project No. 102003Book No. 5576TITLE StudyFrom Page No. 7

REDACTED

Filename:

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Study No.

Treatment	Lot No.	PEGs per G-CSF	Daily Dose (mg/kg)	Group	Animals per Group	Inject. Volume (mL)	Average Weight (kg)	Dosing Conc. (mg/mL)	Nominal Dosing Solution (mL)	Actual Dosing Solution (mL)	Number of Vials	G-CSF (mg)	Total G-CSF (mg)
Vehicle	NA	NA	NA	1	28	0.1	0.1	NA	2.8	5.0	1	NA	NA
rhu-G-CSF	636JOA	0	0.1	2	35	0.1	0.1	0.10	3.5	5.0	1	0.500	
PEG-rhu-G-CSF	4656-71	1	0.1	3	35	0.1	0.1	0.10	3.5	5.0	1	0.500	
PEG-rhu-G-CSF	4656-72	2	0.1	4	35	0.1	0.1	0.10	3.5	5.0	1	0.500	1.500
Totals =					133				13.3	20	4		

Animals: Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule: Single s.c. dose on day 1 (M)

Sacrifice Schedule: Four animals from group 1 will be bled and sacrificed at intervals of 0.5, 1, 1.5, 2, 4, 7 and 10 days following dosing.

Five animals from each of groups 2-4 will be bled and sacrificed at intervals of 0.5, 1, 1.5, 2, 4, 7 and 10 days following dosing.

Injection Notes: Use one vial for each treatment group
 Fill a 1-mL syringe, and inject up to ten hamsters (0.1 mL per animal)
 No need to change syringe needles between hamsters within a single group
 Vials are overfilled by 1.5 mL to allow for losses in filling syringes

Vehicle: 10 mM NaOAc, 5 % (w/v) mannitol, 0.004 % (w/v) Tween 80, pH 4.0

Volume per vial: 5.0 mL

PEG: SS-PEG = PEG succinimidyl succinate, DDI Pharmaceuticals, Inc.
 Lot no. PD-498-KIC (Mn = 47,000)

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To Page No. 8

Recorded by

Christine Farnan

|||

TITLE StudyProject No. 102003
Shipment to Nobel Research Book No. 5576

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From Page No. 4

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Materials: Standard GCSF buffer: 10 mM NaOAc, 5% Mannitol, 0.01% Tween 80, pH 4.0

GCSF lot 636J0A, 0.3 mg/ml in Standard GCSF buffer pH 4.0

~~WFF~~ Mono-Pegylated GCSF lot 5740-5 0.1 mg/ml in Standard GCSF buffer

Di-Pegylated GCSF lot 5740-6 0.1 mg/ml in Standard GCSF buffer

Tri-Pegylated GCSF lot 5740-7 0.1 mg/ml in Standard GCSF buffer

5cc Sterile Pyrogen Free Sample Vials

13 mm West rubber seals

13 mm Flip top crimp seals

Gelman Filter, 2 um sterile Acrodisc, product no. 4192

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Procedure: Group 1 - filtered 4ml of Standard GCSF buffer into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Group 2 - added 4.00ml of Standard GCSF buffer to 2.00ml of GCSF lot 63670A to give a 1 mg/ml solution. Filtered 4ml of this solution into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Group 3 - filtered 4ml of Mono-Pegylated GCSF at 1 mg/ml into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Group 4 - filtered 4ml of Di-Pegylated GCSF at 1 mg/ml into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Group 5 - filtered 4ml of Tri-Pegylated GCSF at 1 mg/ml into 5ml injection vial under aseptic conditions. Sealed vial w/ 13mm rubber seal and 13mm flip top crimp seal.

Vials were labeled and packaged on wet ice and shipped to Nobel Research on 3/5/92. Procedure done same day as shipment.

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Project No. 102003

Book No. 5576

11

TITLE Study

From Page No. _____

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Filename:

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Study No. _____

Treatment	Lot No.	PEGs per G-CSF	Daily Dose (mg/kg)	Animals per Group	Inject. Volume (mL)	Average Weight (kg)	Dosing Conc. (mg/mL)	Nominal Dosing Solution (mL)	Actual Dosing Solution (mL)	Number of Vials	G-CSF (mg)	Total G-CSF (mg)
Vehicle	NA	NA	NA	1	24	0.1	0.1	NA	2.4	3.5	NA	NA
rhu-G-CSF	636JOA	0	0.1	2	24	0.1	0.1	0.10	2.4	3.5	0.350	
PEG-rhu-G-CSF	5740-5	1	0.1	3	24	0.1	0.1	0.10	2.4	3.5	0.350	
PEG-rhu-G-CSF	5740-6	2	0.1	4	24	0.1	0.1	0.10	2.4	3.5	0.350	
PEG-rhu-G-CSF	5740-7	2,3	0.1	5	24	0.1	0.1	0.10	2.4	3.5	0.350	1.400
Totals =					120			12	17.5	5		

Animals: Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule: Single s.c. dose on day 1 (M)

Sacrifice Schedule: Four animals from each group will be bled and sacrificed at intervals of 0.5, 1, 1.5, 2, 4, and 7 days following dosing.

Injection Notes: Use one vial for each treatment group
 Fill a 1-mL syringe, and inject up to ten hamsters (0.1 mL per animal)
 No need to change syringe needles between hamsters within a single group
 Vials are overfilled by 1.1 mL to allow for losses in filling syringes

Vehicle: 10 mM NaOAc, 5 % (w/v) mannitol, 0.004 % (w/v) Tween 80, pH 4.0

Volume per vial: 3.5 mL

PEG: SCM-MPEG = Succinimidyl carboxymethylmethoxypolyethylene glycol
 Lot no. OK2-43c (Mn = 6,000)

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P. Z. Allen

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Christine Farrar

From Page No. X

Materials: CON-INF lot #05272, .2mg/ml in PBS pH 7.0

YM 10 43mm membrane, AMICON

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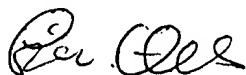
YM 10 43mm membrane, AMICON

SCM-MPEG UCC 84-7

100mM Bicine buffer pH 7.97

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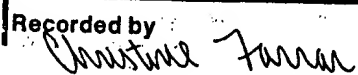


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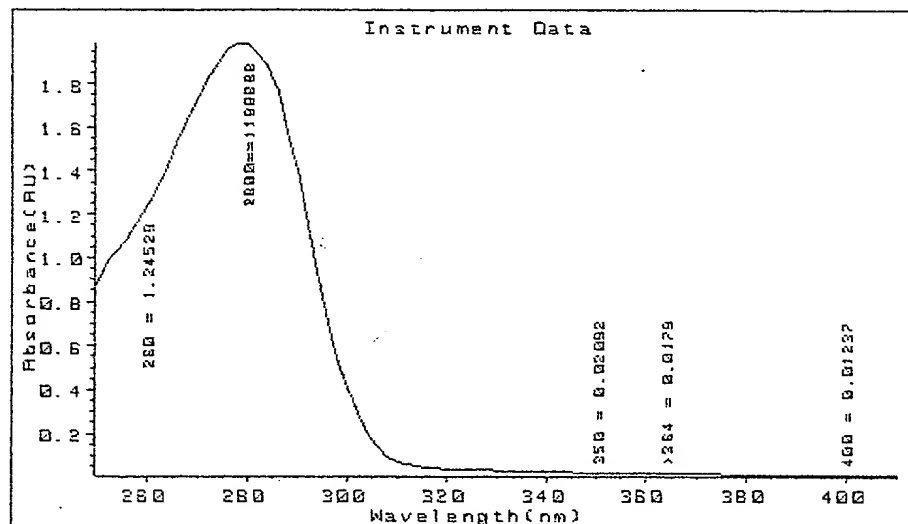
From Page No. 12

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Procedure:

~500 ml of CON-INF lot # 05272 was placed in an Amicon stirred cell and concentrated to < 75 ml using a YM10 membrane. The CON-INF was then buffer exchanged into 100 mM Bicine pH 7.97 via a pressurized reservoir connected to the stirred cell. 450 ml (6 retentate volumes) were exchanged. The CON-INF was then concentrated to ~50 ml and stored at 4°C overnight.

The CON-INF from above was placed in a 50 ml Amicon stirred cell and concentrated to < 10 ml using a YM10 membrane. A 100 μ l aliquot was diluted 10X with 100 mM Bicine buffer and protein concentration was calculated using A_{280} :



GRAPHICS
[LABEL / HARDCOPY]



Values : L280=1.24529 L280=1.97882 L350=0.02092 L400=0.01237
(Stdev) : (0.0024) (0.0043) (0.00013) (0.00015)

Peaks : L280=1.97882 L364=0.01791
(Stdev) : (0.0043) (0.00015)

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$$\frac{1.97882 A_{280} - 0.02092 A_{360}}{1.14 \text{ ml/mg}} \times 10 (\text{dil. factor}) = 17.175 \text{ mg/ml}$$

volume recovered = 6.44 ml

$$(17.175 \text{ mg/ml})(6.44 \text{ ml}) = (10.0 \text{ mg/ml})(x \text{ ml})$$

$$x = 11.06 \text{ ml}$$

$$11.06 - 6.44 = 4.62 \text{ ml}$$

4.62 ml of 100 mM Bicine pH 7.97 were added to the 6.44 ml of CON-INF to give 11.06 ml of 10.0 mg CON-INF/ml in 100 mM Bicine buffer pH 7.97. Stored at 4°C overnight.

7-16-92

SCM-MPEG was weighed into 6 separate 20 ml glass vials. The weight was recorded and the amount of 10 mg/ml CON-INF to be added to each vial to give a 2, 4, 6, 8, 10, and 12 fold molar excess of PEG to CON-INF was calculated. (see table)

ratio PEG:CONI	mg SCM-MPEG	ml 10mg/ml CON-INF	dilution
2	6.0	0.975	3.900
4	11.6	0.943	3.770
6	17.7	0.959	3.835
8	23.2	0.943	3.770
10	29.1	0.946	3.783
12	36.0	0.975	3.900

The appropriate amount of 10.0 mg/ml CON-INF in 100 mM Bicine was added to each vial according to the table above. The reaction mixtures were stirred for 1 hour at room temperature. After 1 hour they were diluted 5X with WFI (see table) and stored at 4°C overnight.

A 0X reaction mixture was also run along side the others as a control. It consisted of 1 ml of unmodified CON-INF in 100 mM Bicine at 10 mg/ml. After 1 hour it was also diluted 5X with WFI and stored overnight.

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from Page No. 1

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Materials: 2x PEG-CON INF from pages 12-14
 4x PEG-CON INF from pages 12-14
 6x PEG-CON INF from pages 12-14
 8x PEG-CON INF from pages 12-14
 10x PEG-CON INF from pages 12-14
 12x PEG-CON INF from pages 12-14
 0x PEG-CON INF from pages 12-14
 ~1ml column of S-Sepharose ff in Pharmacia HR S/S

A - 20 mM NaOAc pH 4.25
 20 mM Citrate pH 3.50

B - 20 mM NaOAc, 1 M NaCl pH 4.25
 20 mM Citrate, 1 M NaCl pH 3.50

Centriprep 10 tubes

PBS buffer pH 7.0 (25 mM NaPhos, .1 M NaCl)

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Christine Farnon

From Page No. 15

Procedure: The S-Sepharose column was equilibrated in 20 mM NaOAc pH 4.25. One ml of the 2X reaction mixture was adjusted to a pH of 4.25 using 1N and .5N HCl. This ml of solution was then loaded onto the column using a Pharmacia FPLC. The PEG-CONINF was eluted using method 0 from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CONINF reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centricon-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution X10 twice. The solution was concentrated until it reached a volume of ~1 ml. The retentate was removed from the centricon-10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 0 BANK 1

0.00 VALUE POS 1.1
0.00 VALUE POS 3.1
0.00 VALUE POS 4.1
0.00 CONC % 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALUE POS 1.2
0.00 CM/ML 0.50
0.00 PORT SET 6.1
1.00 VALUE POS 1.1
5.00 CONC % 0.0
5.00 ML/MIN 1.00
5.00 CONC % 27.5
11.00 CONC % 27.5
11.00 CONC % 100
13.00 CONC % 100
13.00 CONC % 0.0
17.00 PORT SET 6.0
20.00 CM/ML 0.00

CON-01
2X
pH 4.25

FIG. 2

500fs

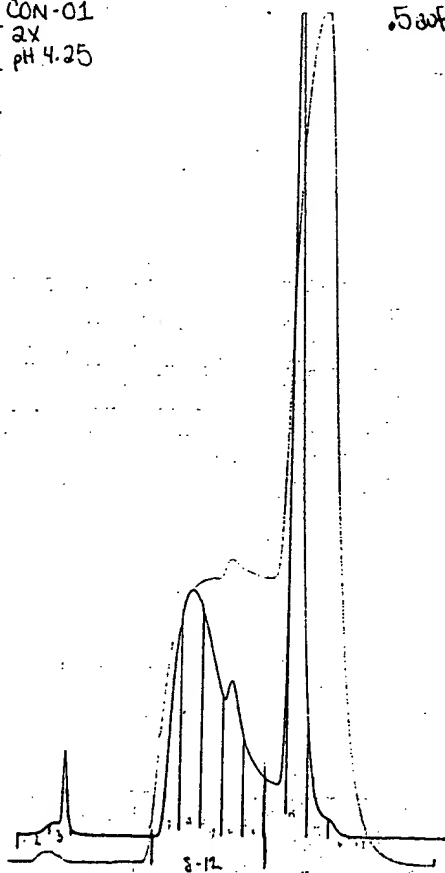
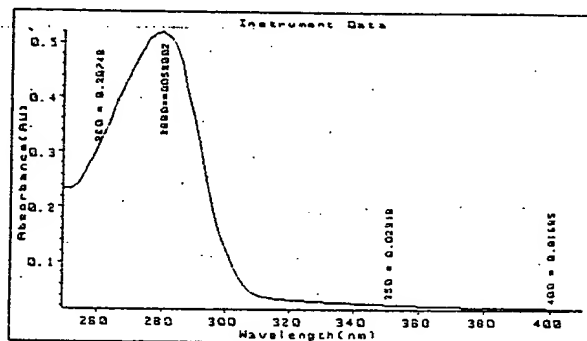


FIG. 3

GRAPHICSD
(LABEL / HARDCOPY)

Values : L260=0.30748 L280=0.52072 L350=0.02318 L400=0.01595
(Stdev) : (0.00012) (0.00018) (0.00011) (0.00010)

$$\frac{0.52072 \text{ AU}_{280} - 0.02318 \text{ AU}_{350}}{1.14 \text{ ml/mg}} = 0.436 \text{ mg/ml}$$

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From Page No. 16

One ml of the 4X reaction mixture was adjusted to a pH of 4.25 using 1N and 5N HCl. This ml of solution was then loaded onto the column using a Pharmacia FPLC. The PEG-CONJEN was eluted using method 0 from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CONJEN 4X reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centricon-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The retentate solution was concentrated until it reached a volume of ~1 ml. The retentate was removed from the centricon-10 and a UVvis scan was taken to determine the protein concentration. (see fig. 3).

FIG. 1

METHOD 0 BANK 1
0.00 VALUE, POS 1.1
0.00 VALUE, POS 3.1
0.00 VALUE, POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALUE, POS 1.2
0.00 CM/ML 0.50
0.00 PORT, SET 6.1
1.00 VALUE, POS 1.1
5.00 CONC %B 0.0
5.00 ML/MIN 1.00
11.00 CONC %B 27.5
11.00 CONC %B 27.5
13.00 CONC %B 100
13.00 CONC %B 100
13.00 CONC %B 0.0
17.00 PORT, SET 6.0
20.00 CM/ML 0.00

FIG. 2

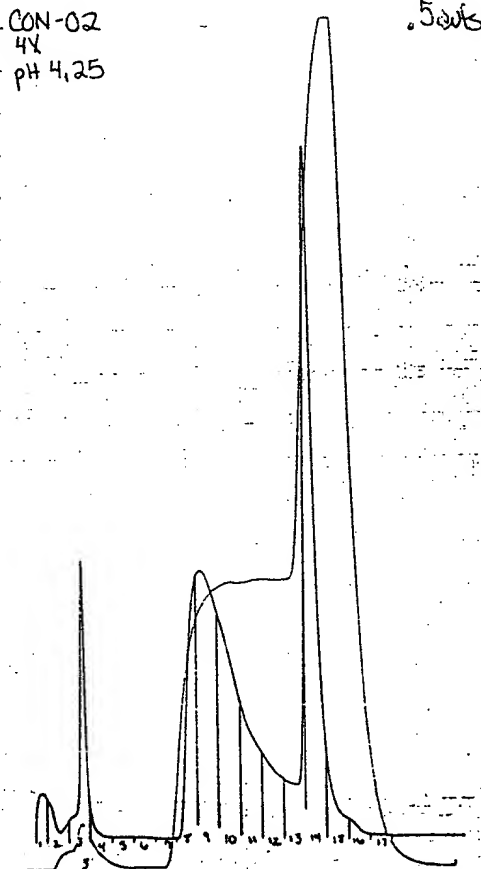
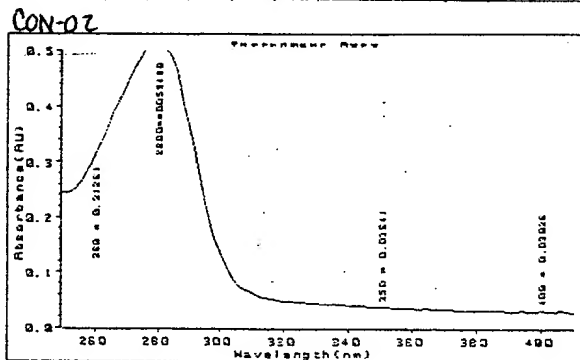


FIG. 3



GRAPHICSD
(LABEL / HARD COPY)

Wavelength (nm)	Absorbance (AU)
260	0.31261
280	0.51469
350	0.03641
400	0.03026

(Stdv) : (0.00024) (0.00034) (0.00019) (0.00018)

0.51469 A₂₈₀ - 0.03641 A₃₅₀ = 0.480 mg/ml
1.14 ml/mg

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One ml of the 6x reaction mixture was adjusted to a pH of 4.25 using 1 N and .5 N HCl. This ml of solution was then loaded onto the column using a Pharmacia FPLC. Then PEG-CON IFN was eluted using method C from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN 6x reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centrprep 10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The solution was concentrated until it reached a volume of ~1 ml. The retentate was removed from the centrprep-10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 0 BANK 1

0.00	VALUE.POS	1.1
0.00	VALUE.POS	3.1
0.00	VALUE.POS	4.1
0.00	CONC %	0.0
0.00	ML/MIN	0.50
0.00	MONITOR	1
0.00	VALUE.POS	1.2
0.00	CM/ML	0.50
0.00	PORT.SET	6.1
1.00	VALUE.POS	1.1
5.00	CONC %	0.0
5.00	ML/MIN	1.00
5.00	CONC %	27.5
11.00	CONC %	27.5
11.00	CONC %	100
13.00	CONC %	100
13.00	CONC %	0.0
17.00	PORT.SET	6.0
20.00	CM/ML	0.00

CON-03
6x
pH 4.25

FIG. 2

5xfs

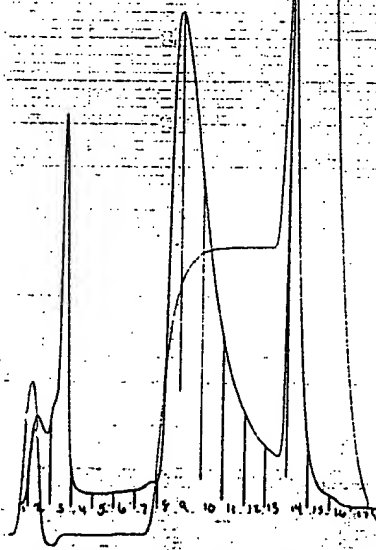
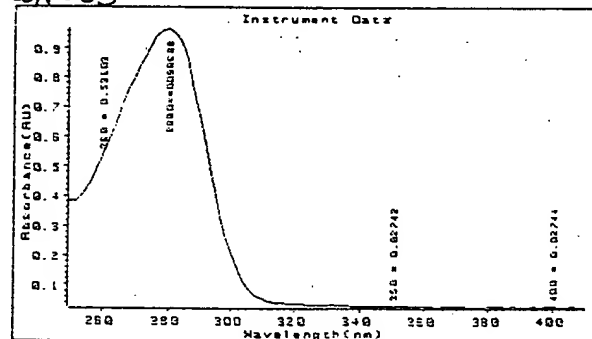


FIG. 3

CON-03

GRAPHICSD
(LABEL / HAPCOPY)

Values : L280=0.93603 L280=0.96683 L350=0.02742 L400=0.02744
(Stdev) : (0.00020) (0.00055) (0.00011) (0.00013)

$$\frac{96683^{A280} - 02742^{A350}}{1.14 \text{ ml/mg}} = .824 \text{ mg/ml}$$

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From Page No. 18

The S-Sepharose column was equilibrated in 20mM Citrate pH 3.50. One ml of the 2X reaction mixture was adjusted to a pH of 3.50 using 1N and 5N HCl. This ml of solution was then loaded onto the column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 1 from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN 2X reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centricon 10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The solution was concentrated until it reached a volume of ~1ml. The retentate was removed from the centricon-10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 1 BANK 1

0.00 VALVE.POS 1.1
0.00 VALVE.POS 3.1
0.00 VALVE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALVE.POS 1.2
0.00 CM/ML 0.50
0.00 PORT.SET 6.1
1.00 VALVE.POS 1.1
5.00 CONC %B 0.0
5.00 ML/MIN 1.00
5.00 CONC %B 50.0
11.00 CONC %B 50.0
11.00 CONC %B 100
13.00 CONC %B 100
13.00 CONC %B 0.0
17.00 PORT.SET 6.0
20.00 CM/ML 0.00

CON-04
2X
pH 3.50

FIG. 2

5.00%

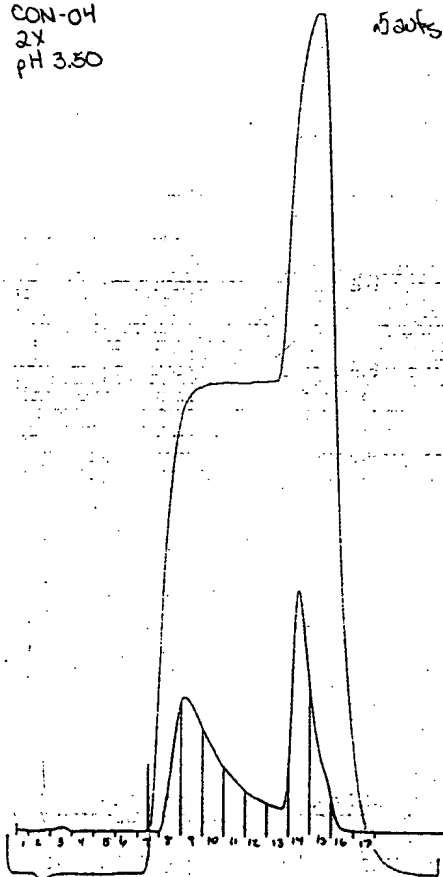
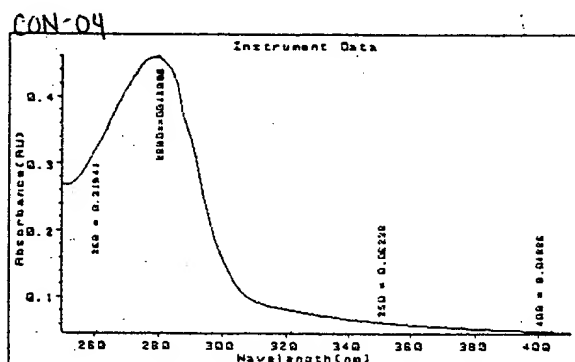


FIG. 3



GRAPHICS
(LABEL / HARD COPY)

Values : L250=0.31941 L280=0.46048 L350=0.06238 L400=0.04886
(Stdev) : (0.00099) (0.00050) (0.00034) (0.00021)

$0.46048 A_{280} = 0.06238 A_{350} = 3.49 \text{ mg/ml}$
 1.14 mg/ml

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Christine Farnan

From Page No. 19

One ml of the 4X reaction mixture was adjusted to a pH of 3.50 using 1N and .5N HCl. This ml of solution was then loaded onto the S-Sepharose column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 0 from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5ml of purified PEG-CON IFN 4X reaction mixture (see fig. 2). This reaction mixture was concentrated to 1ml using an Amicon Centriprep-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The solution was concentrated until it reached a volume of ~1 ml. The retentate was removed from the centriprep-10 and a UV-vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 1 BANK 1

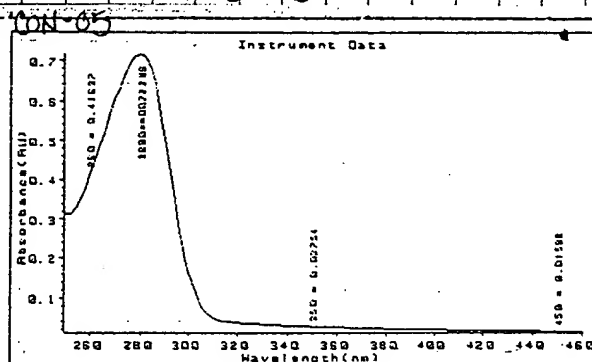
0.00 VALVE.POS 1.1
0.00 VALVE.POS 3.1
0.00 VALVE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALVE.POS 1.2
0.00 CM/ML 0.50
0.00 PORT.SET 6.1
1.00 VALVE.POS 1.1
5.00 CONC %B 0.0
5.00 ML/MIN 1.00
5.00 CONC %B 50.0
11.00 CONC %B 50.0
11.00 CONC %B 100
13.00 CONC %B 100
13.00 CONC %B 0.0
17.00 PORT.SET 6.0
20.00 CM/ML 0.00

CON-05
4X
pH 3.50

FIG. 2



FIG. 3



GRAPHICS
(LABEL / HARD COPY)

Values : L260=0.41637 L280=0.71758 L350=0.02754 L450=0.01598
(Stdev) : (0.00018) (0.00047) (0.00012) (0.00013)

$$\frac{.71758_{A_{280}} - .02754_{A_{350}}}{1.14 \text{ ml/mg}} = .605 \text{ mg/ml}$$

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From Page No. 20

One ml of the lex reaction mixture was adjusted to a pH of 3.50 using 1 N and .5N HCl. This solution was then loaded onto the S-Sepharose column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 1 from CEF (see fig. 1). Fraction 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN (lex reaction mixture) (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centrprep-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The solution was concentrated until it reached a volume of ~1 ml. The retentate was removed from the centrprep-10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 1: BANK 1

0.00 VALVE.POS 1.1
0.00 VALVE.POS 3.1
0.00 VALVE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALVE.POS 1.2
0.00 CM/ML 0.50
0.00 PORT.SET 6.1
1.00 VALVE.POS 1.1
5.00 CONC %B 0.0
5.00 ML/MIN 1.00
5.00 CONC %B 50.0
11.00 CONC %B 50.0
11.00 CONC %B 100
13.00 CONC %B 100
13.00 CONC %B 0.0
17.00 PORT.SET 6.0
20.00 CM/ML 0.00

FIG. 2

CON-OL
6X
pH 3.50

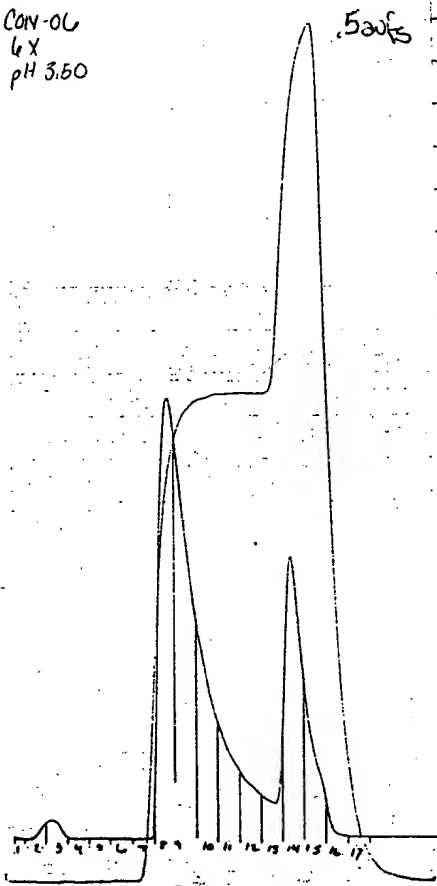
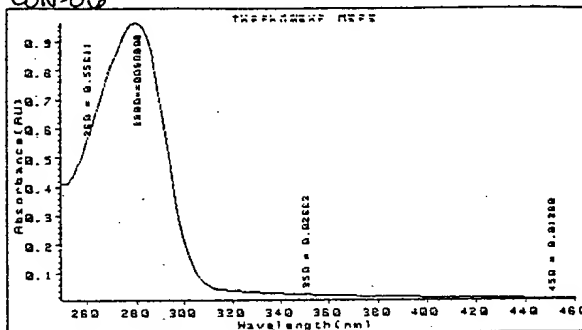
5.50%
5.50%

FIG. 3

CON-OL6

GRAPH1 (CSD
LABEL / HARD COPY)

Values : L260=0.55611 L280=0.97093 L350=0.02663 L450=0.01280
(Stdev) : (0.00022) (0.00062) (0.00010) (0.00013)

$$\frac{97093 A_{280} - 0.02663 A_{350}}{1.14 \text{ ml/mg}} = .828 \text{ mg/ml}$$

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Christine Tarnan

From Page No. 21

One ml of the 8X reaction mixture was adjusted to a pH of 3.50 using 1N and 5N HCl. This ml of solution was then loaded onto the S-Sepharose column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 1 from CEF (see Fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN 8X reaction mixture (see Fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centricon-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution X10 twice. The solution was concentrated to ~1 ml. The retentate was removed from the centricon-10 and a UV/vis scan was taken to determine the protein concentration (see Fig. 3).

FIG. 1

METHOD 1 BANK 1

```

0.00 VALUE.POS 1.1
0.00 VALUE.POS 3.1
0.00 VALUE.POS 4.1
0.00 CONC % 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALUE.POS 1.2
0.00 CM/ML 0.50
0.00 PORT.SET 6.1
1.00 VALUE.POS 1.1
5.00 CONC % 0.0
5.00 ML/MIN 1.00
5.00 CONC % 50.0
11.00 CONC % 50.0
11.00 CONC % 100
13.00 CONC % 100
13.00 CONC % 0.0
17.00 PORT.SET 6.0
20.00 CM/ML 0.00
  
```

FIG. 2

CON-07
8X
pH 3.50

5 ml

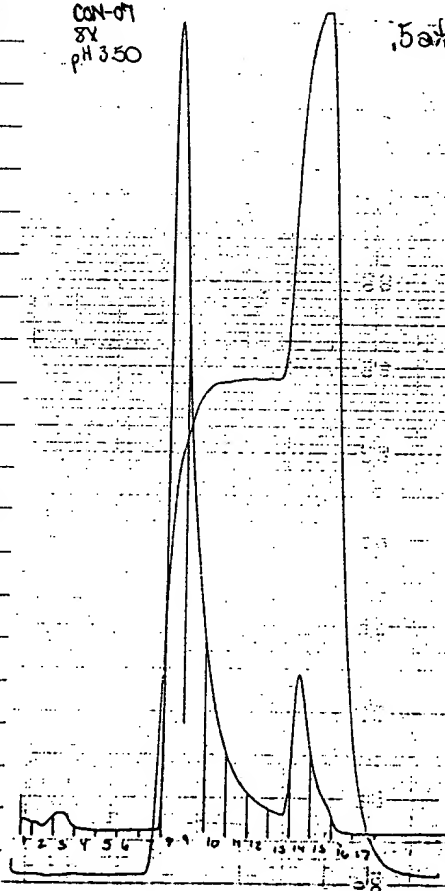
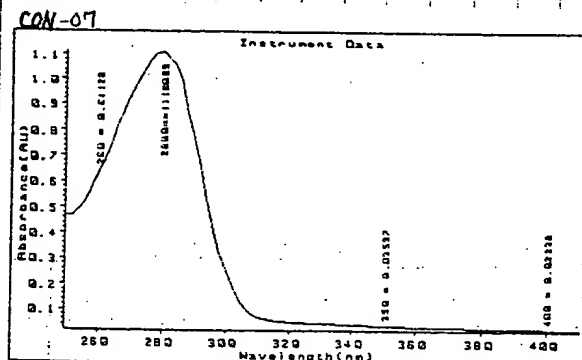


FIG. 3

GRAPHICS
(LABEL / HARDCOPY)

Values: L260=0.54128 L280=1.10953 L350=0.03537 L400=0.02338
(Stdev): (0.0013) (0.0023) (0.00024) (0.00019)

$1.10953^{A_{280}} = 0.03537^{A_{350}} = 0.942 \text{ mg/ml}$

1.14 mg

To Page No. 23

Witnessed & Understood by me,

Date

Invented by

Date

Pro Elec

Recorded by

Christine Tabor

TITLE

REDACTED

From Page No. 22

One ml of the 10X reaction mixture was adjusted to a pH of 3.50 using 1M and .5M HCl. This ml of solution was then loaded onto the Sepharose column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 1 from UFF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN 10X reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centricon-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution X10 twice. The solution was concentrated to ~1 ml. The retentate was removed from the centricon-10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 1 BANK 1

0.00 VALUE.POS 1.1
0.00 VALUE.POS 3.1
0.00 VALUE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 0.50
0.00 MONITOR 1
0.00 VALUE.POS 1.2
0.00 CM/ML 0.50
0.00 PORT.SET 6.1
1.00 VALUE.POS 1.1
5.00 CONC %B 0.0
5.00 ML/MIN 1.00
5.00 CONC %B 50.0
11.00 CONC %B 50.0
11.00 CONC %B 100
13.00 CONC %B 100
13.00 CONC %B 0.0
17.00 PORT.SET 6.0
20.00 CM/ML 0.00

FIG. 2

CON-08
10X
pH 3.50

.5abs

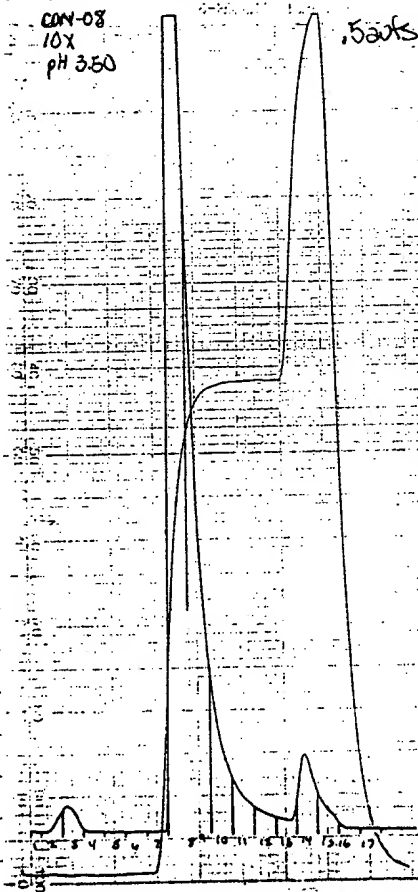
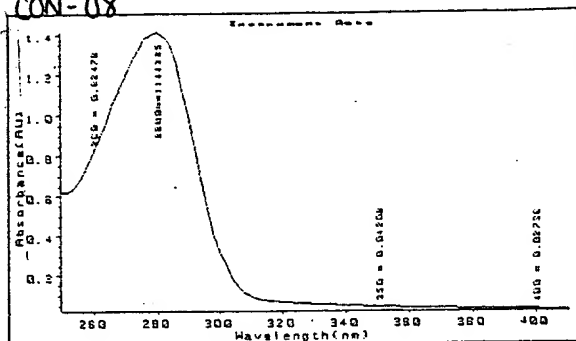


FIG. 3

CON-08



GRAPHICS
CLABEL / HARDCOPY1

Values : L260=0.82478 L280=1.41345 L350=0.04208 L400=0.02736
(Stdev) : (0.00049) (0.0024) (87e-5) (0.00012)

1.41345_{A280} - 0.04208_{A350} = 1.203 mg/ml
1.14 mg/ml

To Page No. 24

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Christine Tarrar

From Page No. 23

One ml of the 12X reaction mixture was adjusted to a pH of 3.50 using 1N and .5 N HCl. This ml of solution was loaded onto the S-Sepharose column using a Pharmacia FPLC. The PEG-CON IFN was eluted using method 1 from CEF (see fig. 1). Fractions 8-12 were collected and pooled to give 5 ml of purified PEG-CON IFN 12X reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centrprep-10 tube, and then buffer exchanged into PBS buffer pH 7.0 by diluting the solution x10 twice. The solution was concentrated to ~1 ml. The retentate was removed from the centrprep 10 and a UV/vis scan was taken to determine the protein concentration (see fig. 3).

FIG. 1

METHOD 1 BANK 1

0.00	VALUE.POS	1.1
0.00	VALUE.POS	3.1
0.00	VALUE.POS	4.1
0.00	CONC %B	0.0
0.00	ML/MIN	0.50
0.00	MONITOR	1
0.00	VALUE.POS	1.2
0.00	CH/ML	0.50
0.00	PORT.SET	6.1
1.00	VALUE.POS	1.1
5.00	CONC %B	0.0
5.00	ML/MIN	1.00
5.00	CONC %B	50.0
11.00	CONC %B	50.0
11.00	CONC %B	100
13.00	CONC %B	100
13.00	CONC %B	0.0
17.00	PORT.SET	6.0
20.00	CH/ML	0.00

FIG. 2

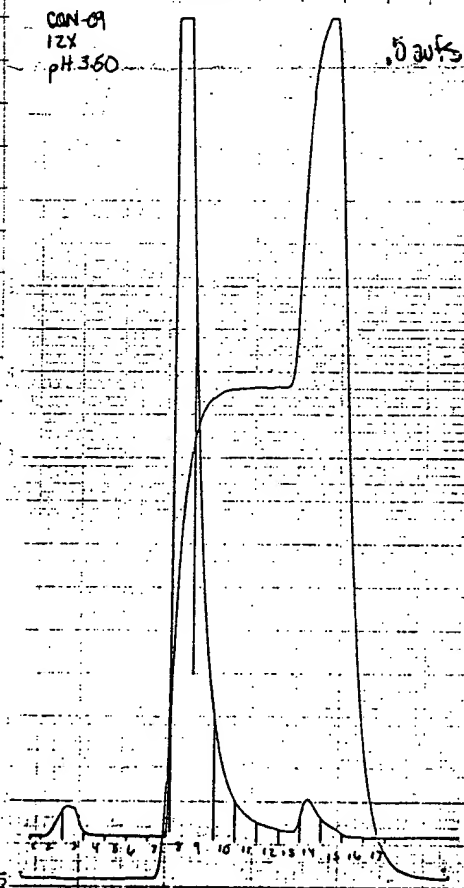
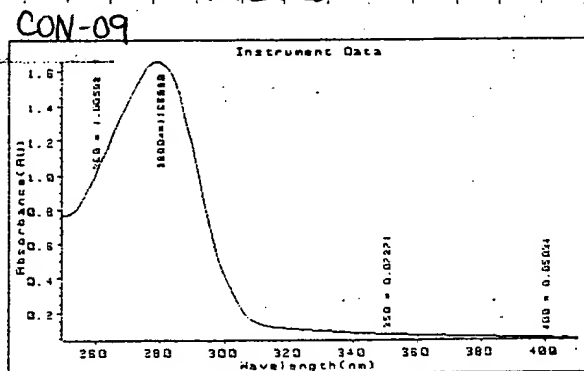


FIG. 3



GRAPH(CSD
(LABEL / HARD COPY)

Values : L260=1.00592 L280=1.65099 L350=0.07271 L400=0.05034
(Stdev) : (0.00050) (0.0025) (0.00010) (0.00013)

$$\frac{1.65099 \text{ AU}_{280} - 0.07271 \text{ AU}_{350}}{1.14 \text{ ml/mg}} = 1.385 \text{ mg/ml}$$

To Page No. 25

Witnessed & Understood by me,

for Clee

Date

Invented by

Date

Recorded by

Christine Turner

|||

TITLE _____

REDACTED

Project No. 150103

Book No. 55710

25

From Page No. 24

One ml of the OX reaction mixture was adjusted to a pH of 3.50 using 1N and 5N HCl. This ml of solution was loaded onto the S-Sepharose column using a Pharmacia FPLC. The ~~FFC~~ unmodified CON-IFN was eluted using method 1 from CEF (see fig 1). Fraction 14 & 15 were collected and pooled to give 2 ml of unmodified CON-IFN OX reaction mixture (see fig. 2). This reaction mixture was concentrated to 1 ml using an Amicon centrprep-10 tube, and then buffer exchanged in PBS buffer pH 7.0 by diluting the solution X10 twice. The solution was concentrated to 1 ml. The retentate was removed from the centrprep 10 and a UV/vis scan was taken to determine protein concentration (see fig. 3).

FIG. 1

METHOD 1 BANK 1
 0.00 VALUE.POS 1.1
 0.00 VALUE.POS 3.1
 0.00 VALUE.POS 4.1
 0.00 CONC %B 0.0
 0.00 ML/MIN 0.50
 0.00 MONITOR 1
 0.00 VALUE.POS 1.2
 0.00 CM/ML 0.50
 0.00 PORT.SET 6.1
 1.00 VALUE.POS 1.1
 5.00 CONC %B 0.0
 5.00 ML/MIN 1.00
 5.00 CONC %B 50.0
 11.00 CONC %B 50.0
 13.00 CONC %B 100
 13.00 CONC %B 100
 17.00 PORT.SET 6.0
 20.00 CM/ML 0.00

FIG. 2

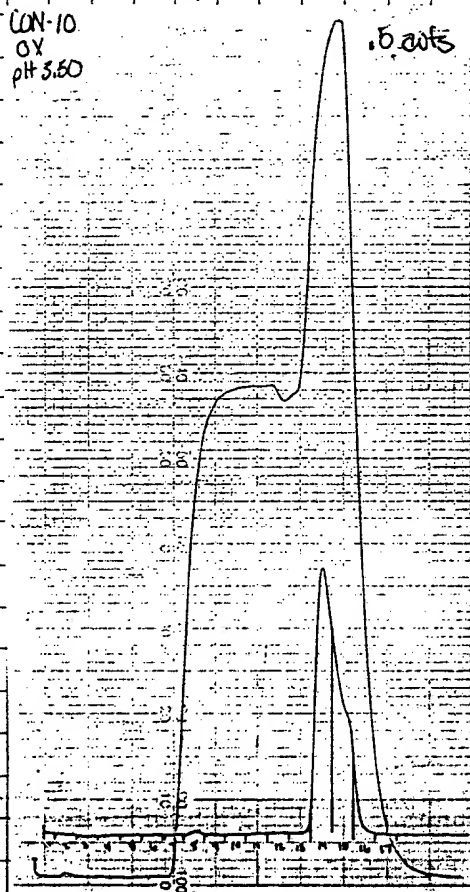
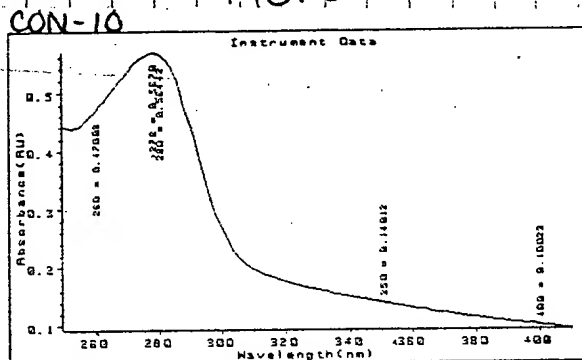


FIG. 3



GRAPHICSDO
 (LABEL / HARD COPY)
 Values : L260=0.47003 L280=0.56442 L350=0.14012 L400=0.10023
 (Stdev) : (0.00017) (0.00025) (94e-5) (88e-5)

56442 Abs - 14012 Abs - 372 mg
 1.14 ml/mg

To Page No. X

Witnessed & Understood by me,

For Oles

Date

Invented by

Recorded by

Christine Farnier

Date

From Page No. X

REDACTED

Materials: Novex "Mark 12" MW STD

CON-IFN lot "05272", 2 mg/ml in PBS pH 7.0

0X CON-IFN CONTROL from page 25
2X PEG-CON IFN from pages 12-14
2X PEG-CON IFN from page 16
4X PEG-CON IFN from pages 12-14
4X PEG-CON IFN from page 17
6X PEG-CON IFN from pages 12-14
6X PEG-CON IFN from page 18
2X PEG-CON IFN from page 19
4X PEG-CON IFN from page 20
6X PEG-CON IFN from page 21
8X PEG-CON IFN from pages 12-14
8X PEG-CON IFN from page 22
10X PEG-CON IFN from pages 12-14
10X PEG-CON IFN from page 23
12X PEG-CON IFN from pages 12-14
12X PEG-CON IFN from page 24

I.S.S. 4-20% Gradient Mini Gels (x2)

Coomassie SOP solutions

To Page No. 27

Witnessed & Understood by me,

P. E. Oles

Date

Invented by

Christine Jarrar

Date

Recorded by

TITLE _____

REDACTED

From Page No. 26

Procedure: SOP for Coomassie stained mini gel

CON-INF-2

Date: _____

Operator: Chris
CON-INF-gel 1

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load μ L	Load μ g
MW STD	1			5.0	
CON INF START	2	0.20	0.15	20.0	3.00
0X CON-INF CONTROL	3	0.37	0.28	10.7	3.00
2X unpurified	4	2.00	1.50	4.0	6.00
2x purified pH 4.25	5	0.44	0.33	18.3	6.00
2x purified pH 3.50	6	0.35	0.26	22.9	6.00
4x unpurified	7	2.00	1.50	4.0	6.00
4x purified pH 4.25	8	0.42	0.31	19.1	6.00
4x purified pH 3.50	9	0.61	0.45	13.2	6.00
6x unpurified	10	2.00	1.50	4.0	6.00
6x purified pH 4.25	11	0.82	0.62	9.7	6.00
6x purified pH 3.50	12	0.83	0.62	9.7	6.00

CON-INF-3

Date: _____

Operator: Chris
CON-INF-gel 2

NB No: _____

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load μ L	Load μ g
MW STD	1			5.0	
CON INF START	2	0.20	0.15	20.0	3.00
0X CON-INF CONTROL	3	0.37	0.28	10.7	3.00
8X unpurified	4	2.00	1.50	4.0	6.00
8X purified pH 3.50	5	0.94	0.71	8.5	6.00
10X unpurified	6	2.00	1.50	4.0	6.00
10X purified pH 3.50	7	1.20	0.90	6.7	6.00
12x unpurified	8	2.00	1.50	4.0	6.00
12X purified pH 3.50	9	1.38	1.04	5.8	6.00

To Page No. X

Witnessed & Understood by me,

Ben Oles

Date

Invented by

Christine Jones

Recorded by

Date

From Page No. 1

REDACTED

Materials: Biorad MW STD
CON-IFN lot "05272" 2 mg/ml in PBS pH 7.0
0X CON-IFN CONTROL from page 23
2X PEG-CON IFN from pages 12-14
2X PEG-CON IFN from page 16
2X PEG-CON IFN from page 19
4X PEG-CON IFN from pages 12-14
4X PEG-CON IFN from page 17
4X PEG-CON IFN from page 20
6X PEG-CON IFN from pages 12-14
6X PEG-CON IFN from page 18
6X PEG-CON IFN from page 21
8X PEG-CON IFN from pages 12-14
8X PEG-CON IFN from page 22
10X PEG-CON IFN from pages 12-14
10X PEG-CON IFN from page 23
12X PEG-CON IFN from pages 12-14
12X PEG-CON IFN from page 24

100 mM NaPhos, 1.5 M NaCl pH 6.9 in Milli Q water

~~Pharmacia~~ Phenomenex SEC 3000 column #35924

Waters HPLC instrument

To Page No. 29

Witnessed & Understood by me,

For Ellis

Date

Invented by

Christine Turner

Date

Recorded by

From Page No. 28

REDACTED

Procedure:

The Samples were submitted & run as follows:

Request # 940

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: C.F.Protein (Analyte): PEG-CONJENAnalysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: NYColumn: SEC3000 #35924Method: CONSEC

Date Results Reported: _____

Instrument # 31

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	5	055-03	1	STD	25				
2	50	04	2	CON-JEN 05972	26				
3	100	15	2		27				
4	200	06	2		28				
5	81	07	.372	10X CON-JEN CONTR	29				
6	15	08	2	2X unpurified	30				
7	69	09	.426	2X purified pH 4.25	31				
8	86	10	.349	2X purified pH 3.50	32				
9	15	11	2	4X unpurified	33				
10	71	12	.420	4X purified pH 4.25	34				
11	50	13	.665	4X purified pH 3.50	35				
12	15	14	2	6X unpurified	36				
13	36	15	.824	6X purified pH 4.25	37				
14	36	16	.828	6X purified pH 3.50	38				
15	15	17	2	8X unpurified	39				
16	32	18	.942	8X purified	40				
17	15	19	2	10X unpurified	41				
18	25	20	1.203	10X purified	42				
19	15	21	2	12X unpurified	43				
20	22	22	1.385	12X purified	44				
21	5	23	1	STD	45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 30

Witnessed & Understood by me,

Date

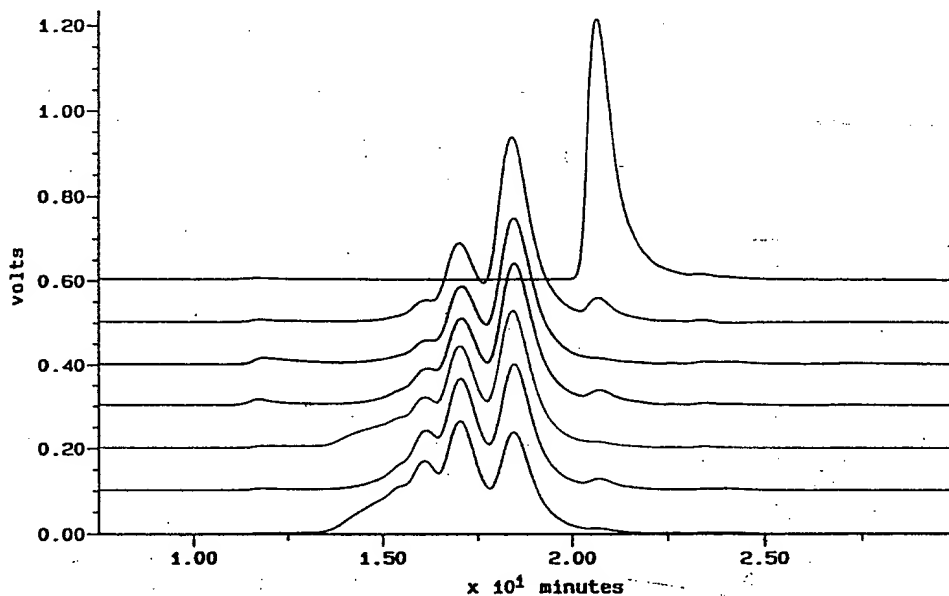
Invented by

Date

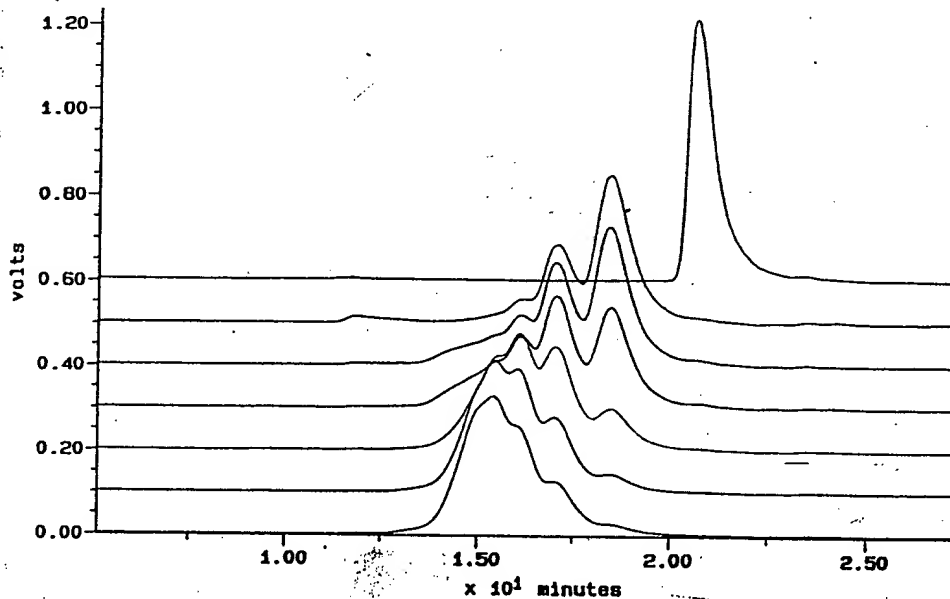
Recorded by

Overlays of resulting Chromatograms:

— 0X RXN
— 2X pH 4.25
— 2X pH 3.50
— 4X pH 4.25
— 4X pH 3.50
— 6X pH 4.25
— 6X pH 3.50



— 0X pH 3.5
— 2X pH 3.5
— 4X pH 3.5
— 6X pH 3.5
— 8X pH 3.5
— 10X pH 3.5
— 12X pH 3.5



Witnessed & Understood by me,

Lee Elie

Date

Invented by

Christina Fusan

Date

Recorded by

|||

Project No. 15203

Book No. 5576

TITLE _____

REDACTED

31

From Page No. 30

Protein Concentration determined by column's calibration:
of different peaks

REQUEST: 940 FROM: CHRIS FARRAR
ANALYSIS: PRO-CON IFF
METHOD: KROMER
COLUMN: SECOD 15024

micrograms AREA

150212.1340 = SLOPE 10.00 1634651
83521.0000 = INTERCEPT 20.00 11954708
0.9988 = R SQUARED 40.00 6277195

FILE	SAMPLE	TIME	AREA	PERCENT	W. (CALCULATED)
CH3_01	EX CONTROL	18.425	75615	0.23	0.0042
		19.542	61486	0.25	0.0042
		20.600	3277277	99.10	0.2592
		23.325	101784	0.31	0.0050
		27.497	3479	0.11	0.0004
		2856319			0.12616
CH3_08	EX UNPURE	16.075	1281875	3.55	0.0444
		16.950	602917	13.34	0.2206
		18.258	19865473	77.49	0.6447
		20.567	2177822	61.10	0.5225
		23.150	343110	6.48	0.1135
		5299497			0.23149
CH3_09	EX PURE 4.25	16.142	721363	5.05	0.0149
		17.000	10101002	22.37	0.0394
		18.392	2894924	64.09	0.2712
		20.467	1362382	7.45	0.0204
		22.472	37194	0.42	0.0052
		23.250	77879	0.42	0.0054
		4515491			0.0276
CH3_10	EX PURE 1.50	16.167	3671671	7.12	0.0144
		17.054	10206010	27.04	0.0738
		18.442	26136495	64.38	0.1813
		22.550	59452	0.16	0.0040
		23.175	227761	0.80	0.0047
		24.154	256115	0.68	0.0045
		3780004			0.2382

CH3_11	EX UNPURE	16.090	615467	11.31	0.0261
		16.943	877024	13.29	0.0258
		18.400	1898673	35.38	0.0822
		20.425	1636240	30.65	0.0971
		23.300	303600	7.17	0.0130
		3248534			0.2352
CH3_12	EX PURE 4.25	16.143	479286	11.43	0.0371
		17.067	1316754	28.18	0.1020
		18.450	2301344	54.88	0.2080
		20.717	199645	4.75	0.0109
		23.242	36130	0.09	0.0075
		23.433	154265	0.38	0.0063
		24.150	122343	0.29	0.0067
		4331187			0.2354
CH3_13	EX PURE 1.50	16.008	220404	0.56	0.0075
		16.117	3833843	21.49	0.0132
		17.067	1370428	28.48	0.1175
		18.437	2304219	67.85	0.2354
		20.575	54442	0.11	0.0044
		22.435	6529	0.14	0.0021
		23.400	177661	0.37	0.0087
		4817129			0.4003
CH3_14	EX UNPURE	16.075	1227640	22.50	0.0504
		17.008	1377047	22.44	0.0505
		18.450	1297747	22.36	0.1400
		20.475	164233	15.01	0.0245
		23.250	429294	7.79	0.0157
		5468491			0.23715
CH3_15	EX PURE 4.25	16.133	360772	0.56	0.0104
		16.150	807004	13.50	0.0152
		17.042	1501304	32.28	0.0522
		18.450	1986508	62.97	0.2542
		20.475	163051	1.52	0.0148
		22.542	36384	0.16	0.0040
		23.454	129430	0.28	0.0130
		24.042	284258	0.41	0.0082
		24.975	26778	0.13	0.0044
		4651702			0.0447
CH3_16	EX PURE 1.50	16.000	80238	0.13	0.0038
		16.002	1500048	21.44	0.0273
		17.000	1375495	35.36	0.0871
		18.442	3627226	31.42	0.2847
		20.417	801127	1.37	0.0032
		22.550	30207	0.25	0.0036
		23.403	137971	0.28	0.0030
		24.025	172820	0.26	0.0022
		4699423			0.0772

CH3_17	EX UNPURE	17.775	57962	0.10	0.0044
		18.500	1328230	21.88	0.3013
		19.075	1256454	34.48	0.5351
		17.002	1405271	25.71	0.5960
		18.500	954742	16.34	0.3469
		20.700	154194	2.82	0.0222
		23.275	479022	8.44	0.1754
		5642149			0.2627
CH3_18	EX PURE	17.867	137515	0.24	0.0149
		18.092	2641713	54.54	0.3363
		17.033	1457797	29.88	0.2809
		18.475	2220152	14.80	0.1229
		22.547	25143	0.07	0.0166
		23.454	104671	0.22	0.0132
		24.050	123803	0.25	0.0148
		4879193			0.2577
CH3_19	EX UNPURE	15.408	2221645	39.22	0.3444
		16.067	1306981	21.26	0.5475
		17.000	3042574	18.42	0.8259
		18.497	485213	6.23	0.1897
		20.642	620077	1.10	0.0093
		23.275	3330481	9.77	0.3205
		5641214			0.2456
CH3_20	EX PURE	15.454	2220156	45.44	0.5712
		16.050	1365194	22.44	0.3413
		17.002	3911129	26.38	0.3039
		18.475	231949	5.98	0.0099
		22.600	1596	0.04	0.0021
		23.467	154243	0.21	0.0161
		5641154			0.2385
CH3_21	EX UNPURE	15.175	2891310	49.53	1.2221
		16.017	1340590	20.05	0.4693
		16.992	7500222	13.13	0.2959
		18.554	233244	6.22	0.1139
		23.325	603443	10.80	0.2357
		5645449			0.2489
CH3_22	EX PURE	15.400	2719548	57.17	0.7978
		16.067	1320599	24.71	0.3257
		17.043	683444	14.40	0.1822
		18.492	185472	1.48	0.0248
		22.410	43016	0.29	0.0029
		23.467	96959	0.20	0.0023
		24.092	125604	0.29	0.0021
		4757827			0.1445

Comparison of Di + Mono Regulated peaks:

REQUEST: 940 FROM: CHRIS FARRAR
ANALYSIS: PRO-CON IFF
METHOD: KROMER
COLUMN: SECOD 15024

micrograms AREA

150212.1340 = SLOPE 10.00 1634651
83521.0000 = INTERCEPT 20.00 11954708
0.9988 = R SQUARED 40.00 6277195

FILE	SAMPLE	TIME	AREA	PERCENT	W. (CALCULATED)
CH3_08	EX PURE 4.25	16.142	721363	22.37	0.0294
		17.000	10101002	64.09	0.2712
		18.392	2894924	7.45	0.0204
		20.467	1362382	0.42	0.0052
		22.472	37194	0.42	0.0054
		23.250	77879	0.42	0.0054
CH3_10	EX PURE 1.50	16.167	3671671	7.12	0.0144
		17.054	10206010	27.04	0.0738
		18.442	26136495	64.38	0.1813
		22.550	59452	0.16	0.0040
		23.175	227761	0.80	0.0047
		24.154	256115	0.68	0.0045
CH3_12	EX PURE 4.25	16.143	479286	11.43	0.0371
		17.067	1316754	28.18	0.1020
		18.450	2301344	54.88	0.2080
		20.717	199645	4.75	0.0109
		23.242	36130	0.09	0.0075
		23.433	154265	0.38	0.0063
CH3_13	EX PURE 1.50	16.008	220404	0.56	0.0075
		16.117	3833843	21.49	0.0132
		17.067	1370428	28.48	0.1175
		18.437	2304219	67.85	0.2354
		20.575	54442	0.11	0.0044
		22.435	6529	0.14	0.0021
CH3_14	EX UNPURE	16.075	1227640	22.50	0.0504
		17.008	1377047	22.44	0.0505
		18.450	1297747	22.36	0.1400
		20.475	164233	15.01	0.0245
		23.250	429294	7.79	0.0157
		5468491			0.23715
CH3_15	EX PURE 4.25	16.133	360772	0.56	0.0104
		16.150	807004	13.50	0.0152
		17.042	1501304	32.28	0.0522
		18.450	1986508	62.97	0.2542
		20.475	163051	1.52	0.0148
		22.542	36384	0.16	0.0040
CH3_16	EX PURE 1.50	16.000	80238	0.13	0.0038
		16.002	1500048	21.44	0.0273
		17.000	1375495	35.36	0.0871
		18.442	3627226	31.42	0.2847
		20.417	801127	1.37	0.0032
		22.550	30207	0.25	0.0036
CH3_18	EX PURE	17.867	137515	0.24	0.0149
		18.092	2641713	54.54	0.3363
		17.033	1457797	29.88	0.2809
		18.475	2220152	14.80	0.1229
		22.547	25143	0.07	0.0166
		23.454	104671	0.28	0.0132
CH3_19	EX UNPURE	15.408	2221645	39.22	0.3444
		16.067	1306981	21.26	0.5475
		17.000	3042574	18.42	0.8259
		18.497	485213	6.23	0.1897
		20.642	620077	1.10	0.0093
		23.275	3330481	9.77	0.3205
CH3_20	EX PURE	15.454	2220156	45.44	0.5712
		16.050	1365194	22.44	0.3413
		17.002	3911129	26.38	0.3039
		18.475	231949	5.98	0.0099
		22.600	1596	0.04	0.0021
		23.467	154243	0.21	0.0161
CH3_21	EX UNPURE	15.175	2891310	49.53	1.2221
		16.017	1340590	20.05	0.4693
		16.992	7500222	13.13	0.2959
		18.554	233244	6.22	0.1139
		23.325	603443	10	
		5641154			

From Page No. 31

Molecular wt. calculated from the Biorad Std.'s peak times on the column

REDACTED

CIS3_13 4X PURE 3.50

11.808	0.56	2160401	12128
16.117	22.49	179333	40332
17.017	28.48	106627	30370
18.417	47.85	47494	22725
20.575	0.11	13651	15
22.533	0.14	4404	6
23.400	0.37	2669	10

REQUEST: #940

FROM: CHRIS FARRAR

SUBMITTED:

ANALYTE: PEG-CON IFN

REPORTED:

METHOD: 2CONSEC

COLUMN: SEC3000 #35924

CIS3_15 6X PURE 4.25

11.733	0.56	2256058	12624
16.150	19.50	175913	34305
17.042	32.28	105098	33923
18.450	42.97	46588	20019
20.675	3.52	12884	453
22.542	0.16	4383	7
23.458	0.28	2581	7
24.042	0.61	1843	11
26.975	0.12	338	0

MW std log MW Retention

670000

-3.9860 = SLOPE
37.0576 = INTERCEPT
0.9966 = R SQUARED

158000

44000

17000

1350

5.1987

4.6435

4.2304

16.28

18.68

20.12

CIS3_16 6X PURE 3-50

11.900	0.18	2048977	3744
16.092	32.34	181942	58839
17.033	31.65	105606	33420
18.442	33.62	46813	15738
20.617	1.37	13326	183
22.525	0.21	4425	9
23.433	0.28	2619	7
24.025	0.36	1860	7

FILE	SAMPLE	TIME	PERCENT	MW	Apparent MW
CIS3_09	2X PURE 4.25	16.142	5.05	176762	8931
		17.000	22.37	107659	24084
		18.392	64.09	48185	30881
		20.667	7.45	12947	964
		22.492	0.42	4511	19
CIS3_10	2X PURE 3.50	23.350	0.62	2748	17
					64896

CIS3_18 8X PURE

11.867	0.24	2088814	5104
16.092	54.54	181942	99233
17.033	29.88	105606	31551
18.475	14.80	45920	6795
22.567	0.07	4320	3
23.458	0.21	2581	6
24.050	0.25	1834	5

CIS3_12	4X PURE 4.25	16.167	7.12	174227	12406
		17.058	27.08	104091	28185
		18.442	64.38	46813	30137
		22.550	0.14	4362	6
		23.475	0.60	2556	15
		24.158	0.68	1723	12

CIS3_20 10X PURE

15.458	45.44	262315	119202
16.050	27.84	186374	51891
17.025	20.38	106115	21627
18.475	5.98	45920	2746
22.600	0.04	4238	2
23.467	0.31	2569	8

CIS3_12	4X PURE 4.25	16.183	11.43	172558	19723
		17.067	28.18	103591	29192
		18.450	54.88	46588	25568
		20.717	4.75	12578	598
		22.542	0.09	4383	4
		23.433	0.38	2619	10
		24.150	0.29	1731	5

CIS3_22 12X PURE

15.400	57.17	271305	155098
16.067	24.37	184588	44988
17.083	14.40	102599	14779
18.492	3.48	45480	1582
22.633	0.09	4157	4
23.467	0.20	2569	5
24.092	0.29	1790	5

216462

To Page No. X

Witnessed & Understood by me,

Eric Oles

Date

Invented by

Christian J. J. J.

Date

Recorded by

From Page No. 8

REDACTED

Materials: 2% IFN-Assay Medium prepared by Steve Warner 8/7/92

0X CON IFN CONTROL from page 25 (557625)
2X PEG-CON IFN from page 16 (557616)
2X PEG-CON IFN from page 19 (557619)
4X PEG-CON IFN from page 17 (557617)
4X PEG-CON IFN from page 20 (557620)
6X PEG-CON IFN from page 18 (557618)
6X PEG-CON IFN from page 21 (557621)
8X PEG-CON IFN from page 22 (557622)
10X PEG-CON IFN from page 23 (557623)
12X PEG-CON IFN from page 24 (557624)
CON IFN lot # 05272

~~centrifuge tubes~~ 1 ml ependorf tubes - plastic

45a spin-x centrifuge filter units by COSTAR

PBS buffer pH 7.0 lot # 07172 (07172B)

To Page No. 34

Witnessed & Understood by me,

For Elee

Date

Invented by

Christine Turner

Recorded by

Date

REDACTED

From Page No. 33

Procedure: All the samples were diluted to 1 mg/ml using the following ratios:

30ul 557625 : 81.6ul WFI
 30ul 557616 : 100.8ul WFI
 30ul 557619 : 74.7ul WFI
 30ul 557617 : 111.0ul WFI
 30ul 557620 : 151.5ul WFI
 30ul 557618 : 217.2ul WFI
 30ul 557621 : 233.4ul WFI
 30ul 557622 : 252.6ul WFI
 30ul 557623 : 330.9ul WFI
 30ul 557624 : 385.5ul WFI
 30ul 05272 : 30ul WFI
 30ul 071728 :

All the samples were then diluted to 20⁹ ml using the following ratios for each sample:

step #1) 20ul sample : 180ul assay medium
 step #2) 20ul of step #1 : 180ul assay medium
 step #3) 100ul of step #2 : 900ul assay medium
 step #4) 225ul of step #3 : 800ul assay medium

All the samples were then to sterile filtered and submitted to Grace Warner for assay.

CONCURRENCE INTERFERON BIOASSAY
 -ANALYTICAL RECORDS-

INITIATED BY: Chris Factor

SAMPLE	ASSAY	DATE	U/ML	UNITS/Mg	S.D. (U/Mg)
1) 557625	52	8/10/92	8.51E+07	8.51E+08	2.73E+08
2) 557616	52	8/10/92	2.64E+07	2.64E+08	1.61E+08
3) 557619	52	8/10/92	2.44E+07	2.44E+08	7.20E+07
4) 557617	52	8/10/92	1.35E+07	1.35E+08	1.47E+08
5) 557620	52	8/10/92	2.67E+07	2.67E+08	1.61E+08
6) 557618	52	8/10/92	1.37E+07	1.37E+08	7.78E+07
7) 557621	52	8/10/92	9.90E+06	9.90E+07	6.52E+07
8) 557622	52	8/10/92	8.40E+06	8.40E+07	2.80E+07
9) 557623	52	8/10/92	8.70E+06	8.70E+07	2.40E+07
10) 557624	52	8/10/92	1.50E+06	1.50E+07	0
11) 05272	52	8/10/92	2.38E+07	2.38E+08	1.05E+08
12) 071728	52	8/10/92	No Activity	No Activity	NA

Assay Performed By: Grace Warner
 Est. 2000

To Page No. _____

Witnessed & Understood by me,

Rita Oles

Date

Initiated by

Christine Turner

Date

Recorded by

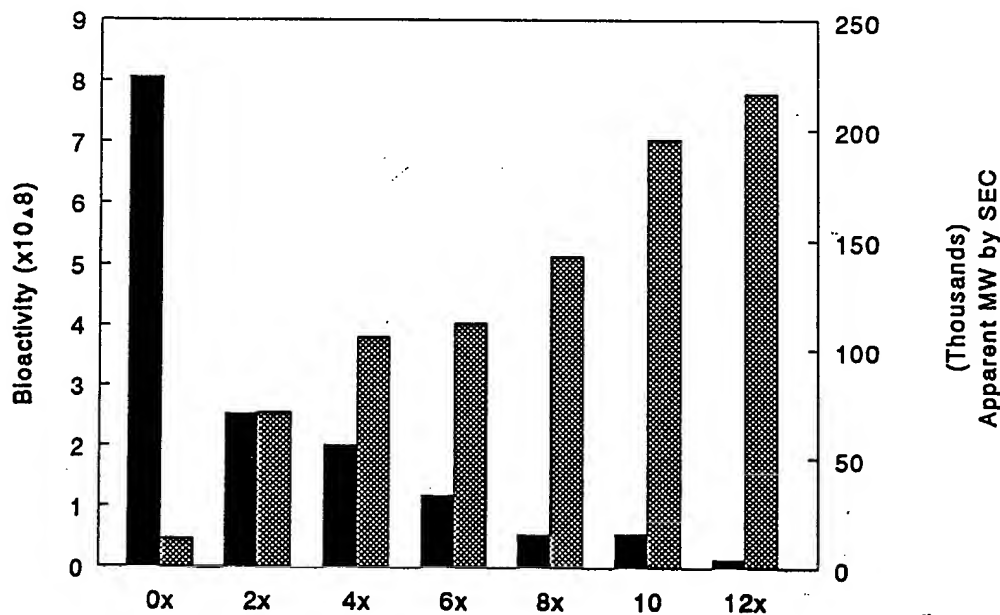
From Page No. 24

Apparent Molecular Weight of the samples is compared to their bioactivity

Bioassay CON-IFN

Date					
#	sample	U/ML	UMG	S.D.(UMG)	APP. MOL. WT.
1	0X	8.51E+07	8.51E+08	2.73E+08	13072
2	2X pH4.25	2.64E+07	2.64E+08	1.61E+08	64896
3	2X pH3.50	2.44E+07	2.44E+08	7.20E+07	70761
4	4X pH4.25	1.35E+07	1.35E+08	1.47E+08	75099
5	4X pH3.50	2.67E+07	2.67E+08	1.51E+08	105587
6	6X pH4.25	1.37E+07	1.37E+08	7.78E+07	101351
7	6X pH3.50	9.90E+06	9.90E+07	8.52E+07	111947
8	8X	5.40E+06	5.40E+07	2.60E+07	142696
9	10X	5.70E+06	5.70E+07	3.40E+07	195475
10	12X	1.50E+06	1.50E+07	0.00E+00	216462
11	05272	2.39E+07	2.39E+08	1.50E+08	13072
12	BUFFER	0.00E+00	0.00E+00	NA	0

Apparent Molecular Weight vs
In Vitro Activity



To Page No. x

Witnessed & Understood by me,

[Signature]

Date

Invented by

[Signature]

Date

Recorded by

From Page No. 1

REDACTED

To Page No. 31

Witnessed & Understood by me,

Ran Ellis

Date

Invented by

Christine Jordan

Date

Recorded by

111

Project No. 150103
Book No. 5576

TITLE _____ 37

From Page No. 36

REDACTED

To Page No. X

Witnessed & Understood by me,

Ben Ellis

Date

Invented by

Christine Finner

Date

Recorded by

From Page No. 1

REDACTED

Materials: GCSF lot #50140 T6702 2.9mg/ml in Mannitol
r-met Hu

Water for Irrigation (WFI) from Baxter pH 3.25

500mM Bicine buffer pH 8.0

YM 10 13mm membrane, AMICON

SCM-MPEG UCC 84-7

To Page No. 39

Witnessed & Understood by me,

Rita E. Lee

Date

Invented by

Christine Jones

Date

Recorded by

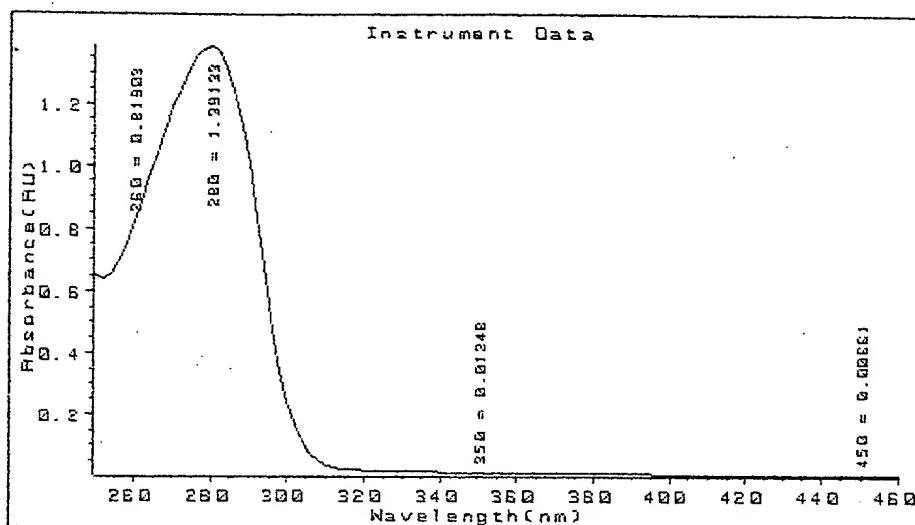
TITLE _____

From Page No. 38

REDACTED

Procedure :

~ 350 ml of GCSF lot #S0140167 was placed in an Amicon stirred cell and concentrated to ~ 50 ml using a YM10 membrane. The GCSF was then buffer exchanged into WFI pH 3.25 via a pressurized reservoir connected to the stirred cell. 300 ml (6 retentate volumes) were exchanged. A 100 ml aliquot was diluted 10X with WFI and its protein concentration was calculated using A_{280} .



GRAPHICSCO
[LABEL / HARDCOPY]

Values : L260=0.81903 L280=1.39133 L350=0.01248 L450=0.00661
(Stdev) : (0.00041) (0.0023) (0.00013) (0.00012)

$$1.39133^{A_{280}} \times 0.86 \text{ mg/ml} \times 10 (\text{dil. factor}) = 16.178 \text{ mg/ml}$$

$$(16.178 \text{ mg/ml}) (70.05 \text{ ml}) = (12.5 \text{ mg/ml}) (x \text{ ml})$$

$$54.12 \quad x = 70.05$$

$$70.05 \text{ ml} - 54.12 \text{ ml} = 15.93 \text{ ml}$$

15.93 ml of WFI pH 3.25 were added to the 54.12 ml of GCSF to give 70.05 ml of 12.5 mg/ml GCSF in WFI pH 3.25. Stored at 4°C overnight.

The protein concentration was checked using A_{280} (see Figure below)

To Page No. 40

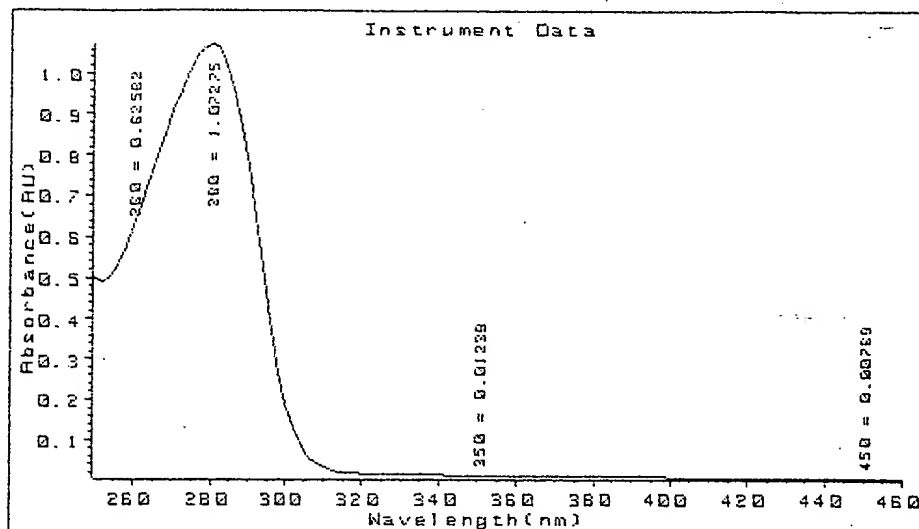
Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 39

GRAPHICS

[LABEL / HARDCOPY]



E

Values : L260=0.62582 L280=1.07275 L350=0.01239 L450=0.00769
 (Stdev) : (0.00033) (0.0010) (93e-6) (0.00010)

$$1.07275 A_{280} / 0.86 \text{ ml/mg} \times 10 (\text{dil factor}) = 12.5 \text{ mg/ml}$$

Six ml of 500mM Bicine was added to 24 ml of the 12.5 mg/ml GCSE solution to give a 10 mg/ml GCSE in 100mM Bicine of 30ml or 300 mg of GCSE. The rest of the 12.5 mg/ml GCSE in WFI pH 3.25 was diluted to 5 mg/ml and stored at 4C.
 (sterile filtered)

0.0630g of SCM-MPEG and 0.1212g of SCM-MPEG was weighed into 2 separate 100ml beakers pyrex. The amount of 10 mg/ml GCSE to be added to each beaker to give 1.5 fold and 3.0 fold molar excess of PEG to GCSE, using at least 100mg of GCSE for each reaction, was calculated:

$$\left[\frac{(0.0630 \text{ g PEG})}{(6000 \text{ mol. wt. PEG})} \right] \times 1.5 \text{ fold} \times 18,800 \text{ mol. wt. GCSE} = 213.6 \text{ g GCSE}$$

or 3.16 ml

To Page No. 41

Witnessed & Understood by me,

For Oles

Date

Invented by

Christine Jamar

Recorded by

Date

From Page No. 40

$$\left[\frac{.1212 \text{ g PEG}}{6000 \text{ mol. wt. PEG}} \right] / 3.0 \text{ fold} \times 18,800 \text{ mol. wt. GCSF} = .1266 \text{ g GCSF} \text{ or } 12.66 \text{ ml}$$

The appropriate amount of 10 mg/ml GCSF in 100 mM Bicine was added to each beaker according to the calculations above. The reaction mixtures were stirred for 1 hour at room temperature. After 1 hour, each reaction mixture was diluted 5X with WFI and adjusted to a pH of 4.0 and then ~~stored~~ sterile filtered and stored at 4C.

To Page No. X

Witnessed & Understood by me,

Ben Oles

Date

Invented by

Christine Farnan

Date

Recorded by

From Page No. X

REDACTED

Materials: ~~G~~ 3.0X SCM-MPEG GCSF, 2 mg/ml in 4.0 WFI, from page 39.
1.5X SCM-MPEG GCSF, 5 mg/ml in 4.0 WFI, from page 39.

20mM NaOAc buffer pH 4.0

20mM NaOAc, 1 M NaCl buffer pH 4.0

1ml Pharmacia column #9147291 Mono-S HP 5/5

Pharmacia FPLC instrument

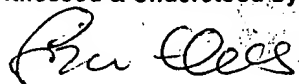
I.S.S. 4-20% Gradient Mini Gels

r-met Hu GCSF lot #S0140T6702 5 mg/ml pH 3.25 WFI
(from page 39)

Comassie SOP solutions

To Page No. 43

Witnessed & Understood by me,



Date

4.27.02

Invented by



Recorded by

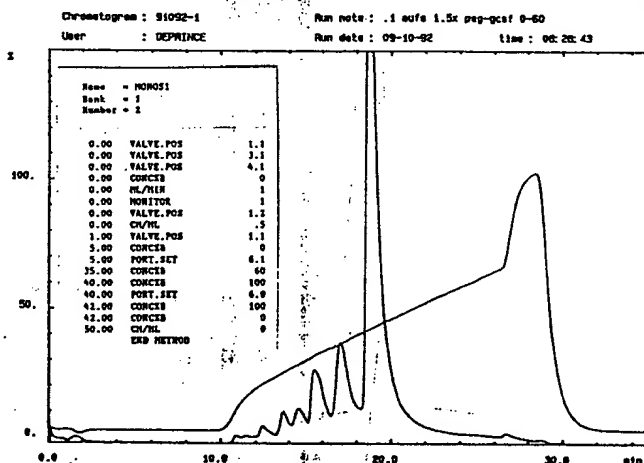
Date

From Page No. 42

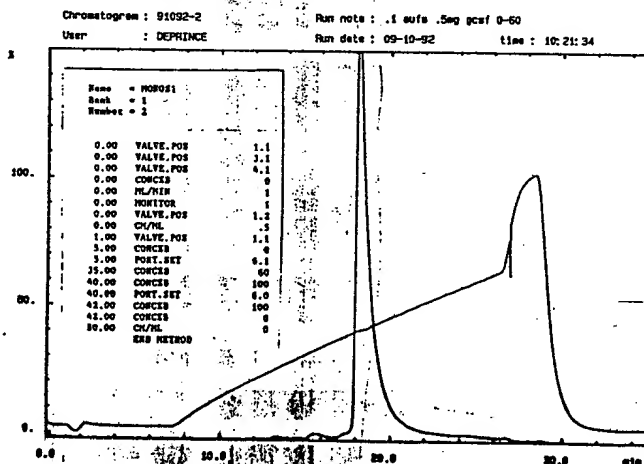
REDACTED

Procedure:

The 1 ml Mono-S column was equilibrated in 20mM NaOAc pH 4.0. 1 mg of the 1.5X PEG-GCSF was loaded onto the column using a Pharmacia FPLC. The PEG-GCSF was eluted using method 2 from CEF:



5mg of the buffer exchange starting material GCSF in 4.0 WFI from page 39 was loaded onto the column and was eluted using the same method 2 from CEF:



To Page No. 44

Witnessed & Understood by me,

For CEF

Date

Invented by

Deprince Farnan

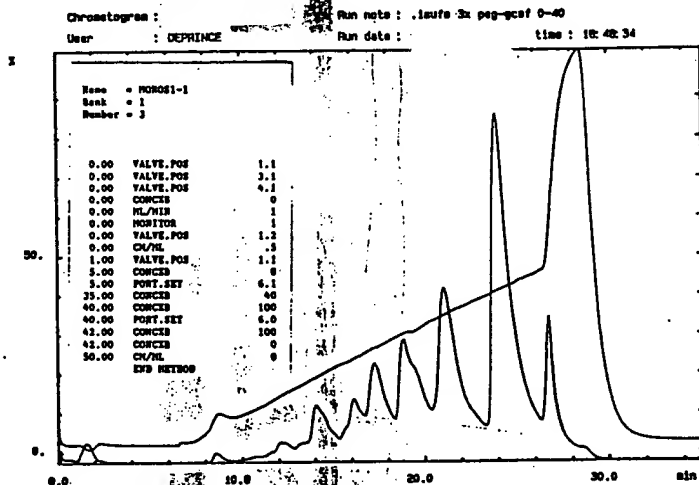
Recorded by

Date

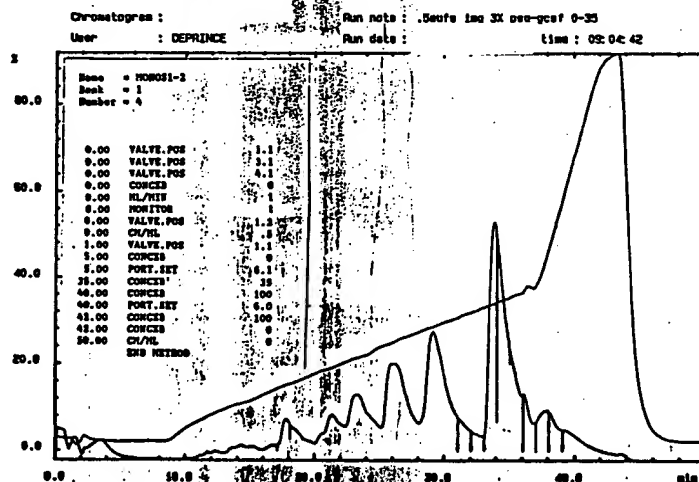
REDACTED

From Page No. 43

Method 2 from CEF was modified and 1mg of the 3.0X PEG-GCSF was loaded onto the column and eluted using method 3 from CEF.



Method 3 was modified and 1mg of the 3.0X PEG-GCSF was loaded onto the column and eluted using method 4 from CEF.

To Page No. 45

Witnessed & Understood by me,

Date

Invented by

Date

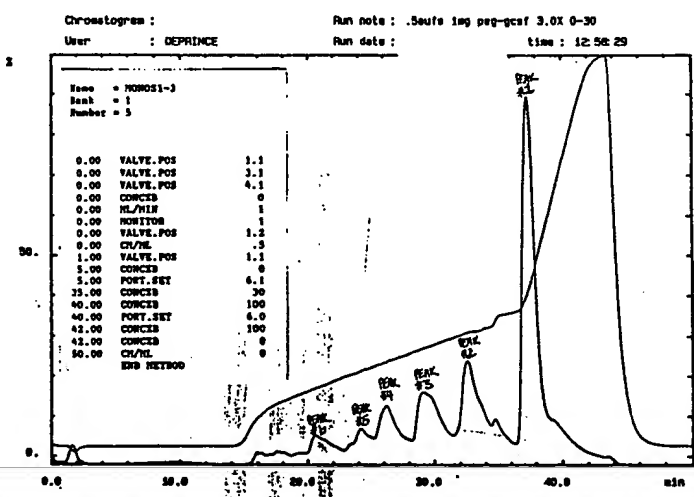
Recorded by

TITLE _____

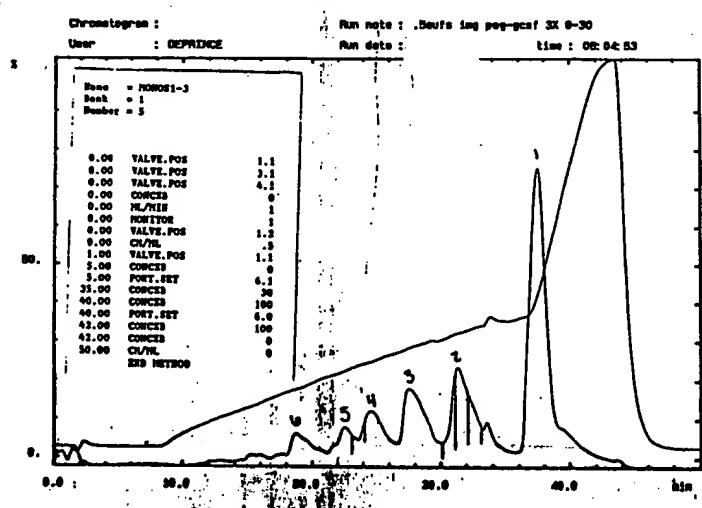
REDACTED

From Page No. 44

Method 4 was modified and 1mg of the 3.0X PEG-GCSF was loaded onto the column and eluted using method 5 from CEF. Fractions from each of the six peaks were collected and pooled separately.



The Method from [redacted] was repeated 3X to elute 3mg more of 3.0X PEG-GCSF. Each time, fractions from the six peaks were collected and pooled w/ their congruent peak from [redacted]



To Page No. 46

Witnessed & Understood by me,
For Cles

Date _____

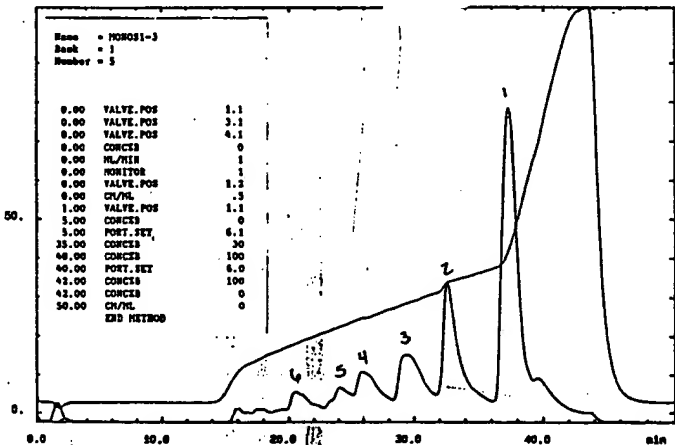
Invented by *Christine Farrow*
Recorded by _____

Date _____

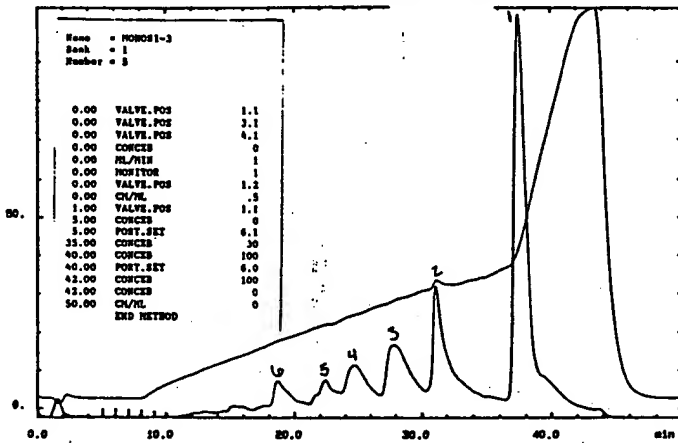
REDACTED

From Page No. 45

Chromatogram : Run note : .5aufr ing peg-gcsf 3X 0-30
User : DEPRINCE Run date : time : 02:34:16

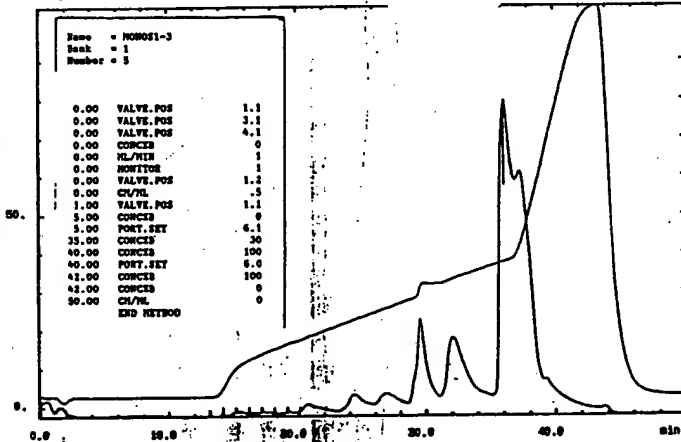


Chromatogram : Run note : .5aufr ing peg-gcsf 3X 0-30
User : DEPRINCE Run date : time : 10:41:41



1 mg of 1.5X PEG-GCSF was loaded onto the column and eluted using method 5 from REF:

Chromatogram : Run note : .5aufr ing peg-gcsf 1.5X 0-30
User : DEPRINCE Run date : time : 09:00:35



To Page No. 47

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

TITLE _____

REDACTED

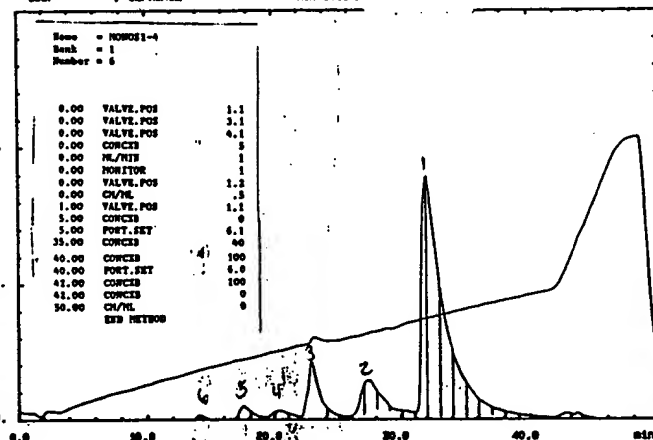
From Page No. 46

Method 5 was modified and 1mg of 1.5X PEG-GCSF was loaded onto the column and eluted using method 6 from CEF. Fractions from the six peaks were collected and pooled separately:

Chromatogram: Run note: .1AUF5 1MG PEG-GCSF 1.5X 0-40
User: DEPRINCE Run date: time: 11:31:20

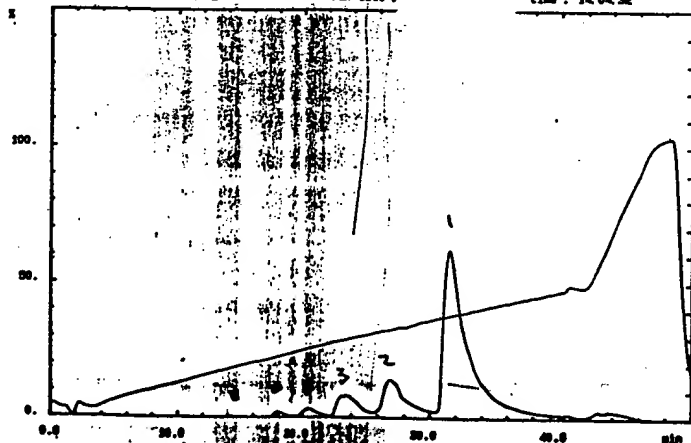
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Bank = 1
Number = 6

0.00	VALVE.POS	1.1
0.00	VALVE.POS	2.1
0.00	VALVE.POS	4.1
0.00	CONCISE	5
0.00	HL/HTS	1
0.00	MONITOR	1
0.00	VALVE.POS	1.2
0.00	CONCISE	5
1.00	VALVE.POS	1.1
5.00	CONCISE	0
5.00	PORT.SET	6.1
35.00	CONCISE	60
40.00	CONCISE	100
40.00	PORT.SET	6.0
41.00	CONCISE	100
41.00	CONCISE	0
50.00	CONCISE	0
50.00	CONCISE	0
50.00	CONCISE	0

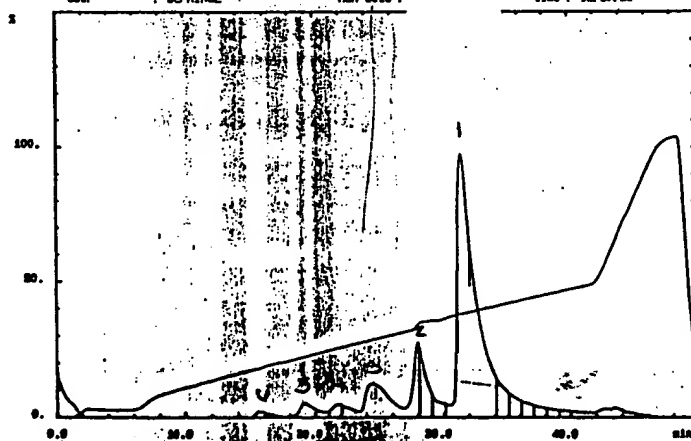


The method from _____ was repeated 6x to elute 1mg more of 1.5X PEG-GCSF. Each time, fractions from the six peaks were collected & pooled with their congruent peak from _____

Chromatogram: Run note: .1AUF5 1MG PEG-GCSF 1.5X 0-40
User: DEPRINCE Run date: time: 14:04:32



Chromatogram: Run note: .1AUF5 1MG PEG-GCSF 1.5X 0-40
User: DEPRINCE Run date: time: 12:57:00



To Page No. 48

Witnessed & Understood by me,

Date _____

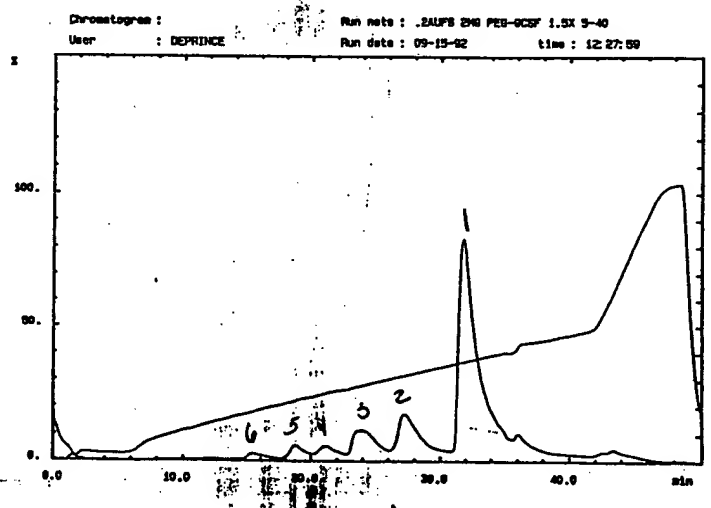
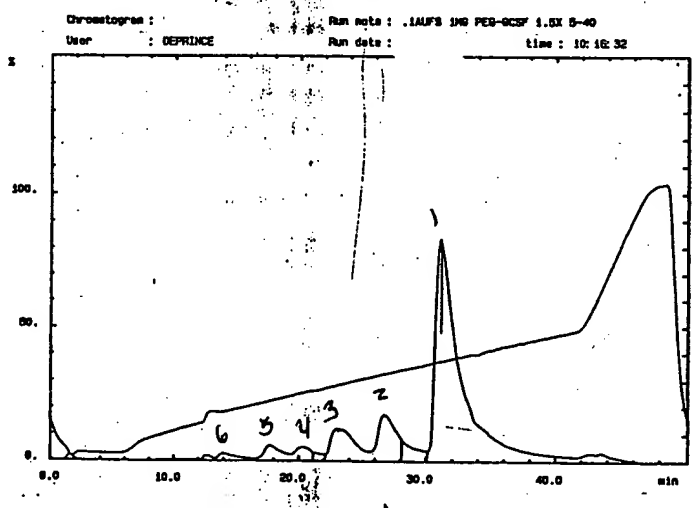
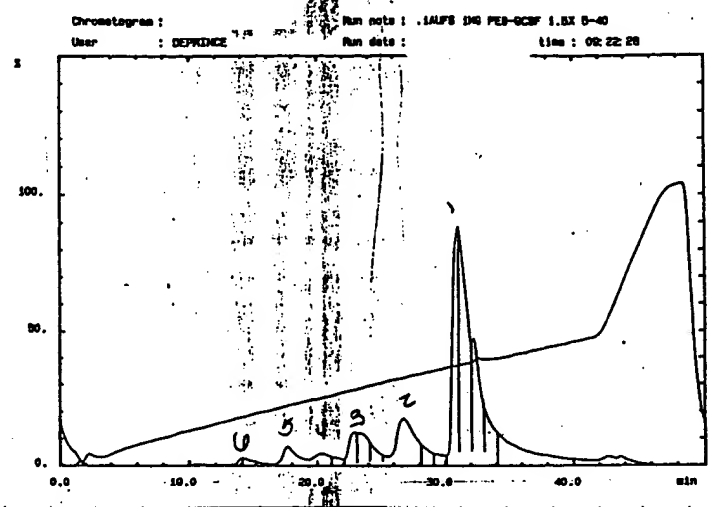
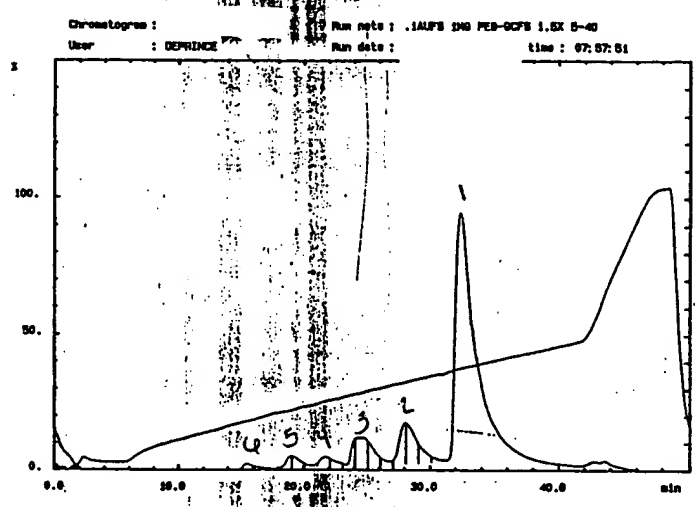
Invented by _____

Date _____

Recorded by _____

From Page No. 47

REDACTED



To Page No. 49

Witnessed & Understood by me,
Ben Oles

Date _____

Invented by
Christine Tabor
Recorded by _____

Date _____

TITLE _____

From Page No. 48

REDACTED

The samples from the six peaks from each reaction mixture were concentrated and run on an SDS/PAGE Gradient Mini Gel using the SOP for Coomassie stained mini gels.

GCSF-10

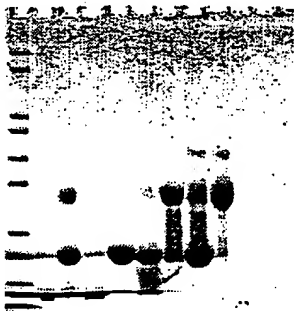
Date: _____
Operator: Chris
PEG-GCSF gel 1

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load μ L	Load μ g
MW STD	1			10.0	
GCSF	2	1.00	0.75	4.0	3.00
PEG-GCSF 3.0X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	1.55	1.16	8.6	10.00
PEAK #2	5	0.65	0.49	20.5	10.00
PEAK #3	6	0.62	0.47	21.4	10.00
PEAK #4	7	1.24	0.93	10.7	10.00
PEAK #5	8	1.00	0.75	13.4	10.00
PEAK #6	9	0.84	0.63	15.9	10.00



GCSF-11

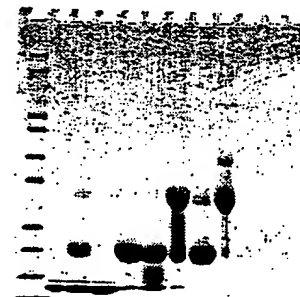
Date: 1/8/92
Operator: Chris
PEG-GCSF gel 2

NB No: _____
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load μ L	Load μ g
MW STD	1			10.0	
GCSF	2	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	1.51	1.14	8.8	10.00
PEAK #2	5	0.64	0.48	20.9	10.00
PEAK #3	6	1.04	0.78	12.8	10.00
PEAK #4	7	0.81	0.61	16.4	10.00
PEAK #5	8	0.92	0.69	14.6	10.00
PEAK #6	9	1.05	0.79	12.7	10.00



To Page No. X

Witnessed & Understood by me,

For Lee

Date _____

Invented by _____

Recorded by _____

Date _____

From Page No. X

REDACTED

Materials: r-met Hu GCSF Id #S014076702 5 mg/ml in WFI (from page 39)
pH 3.25

SCM-MPEG UCC 84-M

500 mM Bicine buffer pH 8.0 in WFI

Centriprep 10 concentrating units, AMICON (x8)

20 mM NaOAc pH 4.0 buffer

20 mM NaOAc, 1 M NaCl pH 4.0 buffer

44 ml SP Sepharose HP column

3.0X SCM-MPEG-GCSF, 2 mg/ml in 4.0 WFI, from page 39

1.5X SCM-MPEG-GCSF, 2 mg/ml in 4.0 WFI, from page 39

Waters HPLC instrument

Phenomenex SEC3000 column #33889

100 mM NaPhos pH 6.9 in Milli Q water

I.S.S. 4-20% Gradient Mini Gels (x2)

Comassie ROP solutions

4.5 cc Nalgene 150 ml filter unit (x2)

To Page No. 51

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

|||

TITLE

From Page No. 50

REDACTED

Procedure:

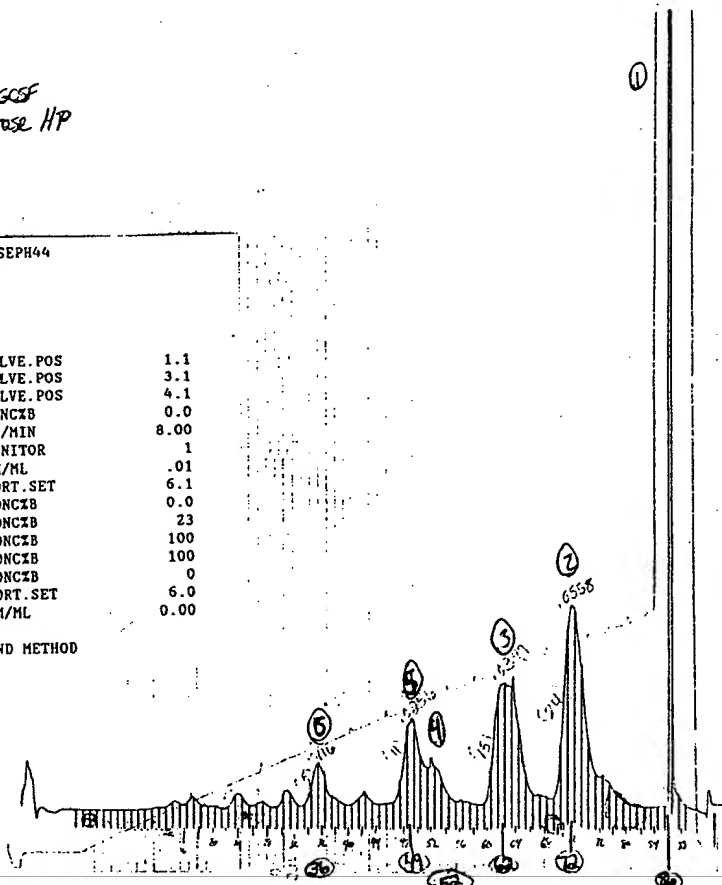
The 44ml SP Sepharose HP column was equilibrated in 20mM NaAc pH 4.0. 44mg of the 1.5X PEG-GCSF from page 39 was loaded onto the column using a Pharmacia FPLC. The PEG-GCSF was eluted using method 0 from CEF. Fractions from each of six peaks were collected & stored at 4C.

file =
44mg 1.5X PEG-GCSF
44ml SP Sepharose HP
AUFs = 0.1

Name = SSEPH44
Bank = 1
Number = 0

0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	VALVE.POS	4.1
0.00	CONCIB	0.0
0.00	ML/MIN	8.00
0.00	MONITOR	1
0.00	CH/ML	.01
132.0	PORT.SET	6.1
132.0	CONCIB	0.0
1454	CONCIB	23
1454	CONCIB	100
1542	CONCIB	100
1542	CONCIB	0
1542	PORT.SET	6.0
1630	CH/ML	0.00

END METHOD



To Page No. 52

Witnessed & Understood by me,

Per Eley

Date

Invented by

Christine Farnum

Recorded by

Date

Project No. 102003Book No. 8014

TITLE _____

2

REDACTED

rom Page No. 2

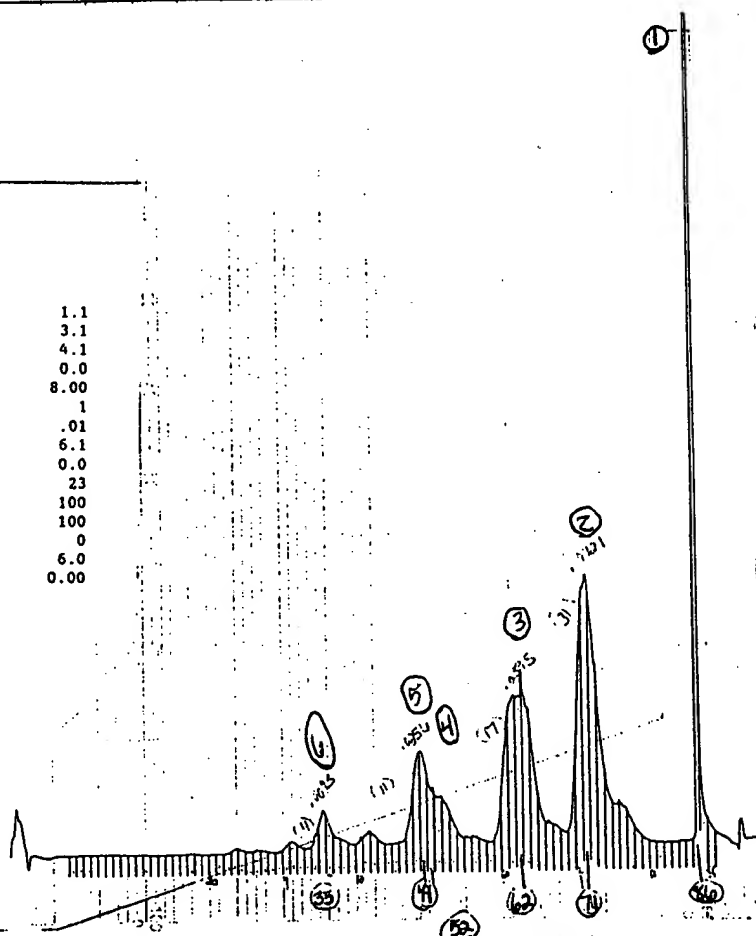
44 mg of 1.5X PEG-GCSF were loaded onto the column and eluted using Method ① from CEF. Fractions from each of the six peaks were collected and pooled with their congruent fraction from _____ and stored at 4°C.

File =
44mg 1.5X PEG-GCSF
44ml SP Sepharose HP
AUFs = 1

Name = SSEPH44
Bank = 1
Number = 0

0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	VALVE.POS	4.1
0.00	CONCXB	0.0
0.00	ML/MIN	8.00
0.00	MONITOR	1
0.00	CH/ML	.01
132.0	PORT.SET	6.1
132.0	CONCXB	0.0
1454	CONCXB	23
1454	CONCXB	100
1542	CONCXB	100
1542	CONCXB	0
1542	PORT.SET	6.0
1630	CH/ML	0.00

END METHOD

To Page No. 55

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

TITLE

From Page No. 52

REDACTED

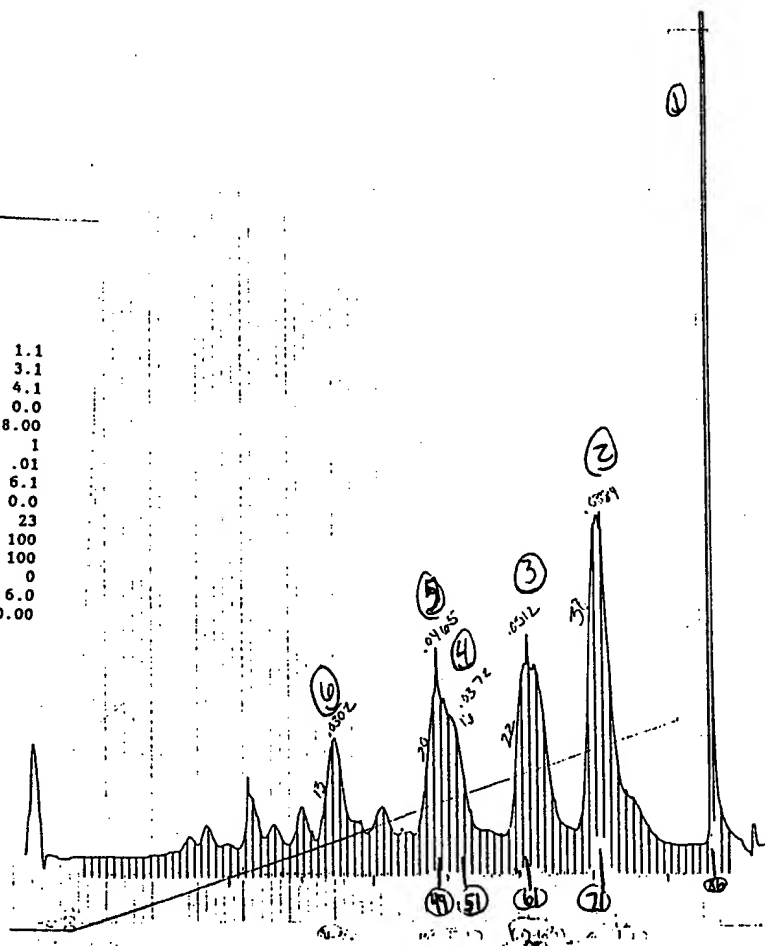
44 mg of 3.0X PEG-GCSF were loaded onto the column and eluted using Method 0 from CEF. Fractions from each of the six peaks were collected and pooled with and stored at 4C.

File =
44mg 3.0X PEG-GCSF
44ml SP Sephadex HP
AUFS = 0.1

Name = SSEPH44
Bank = 1
Number = 0

0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	VALVE.POS	4.1
0.00	CONCIB	0.0
0.00	ML/MIN	8.00
0.00	MONITOR	1
0.00	CM/ML	.01
132.0	PORT.SET	6.1
132.0	CONCIB	0.0
1454	CONCIB	23
1454	CONCIB	100
1542	CONCIB	100
1542	CONCIB	0
1542	PORT.SET	6.0
1630	CM/ML	0.00

END METHOD



To Page No. 54

Witnessed & Understood by me,

R. C. Cles

Date

Invented by

Christine Farnon

Date

Recorded by

Project No. 102003Book No. 5510

TITLE _____

REDACTED

From Page No. 53

50 mg of 3.0X PEG-GCSEF was loaded onto the column ~~the~~ and eluted with method 8 from CEF. Fractions from the six peaks were collected and pooled with their congruent peak fraction from and stored at 4C.

File =

50 mg 3.0X PEG-GCSEF

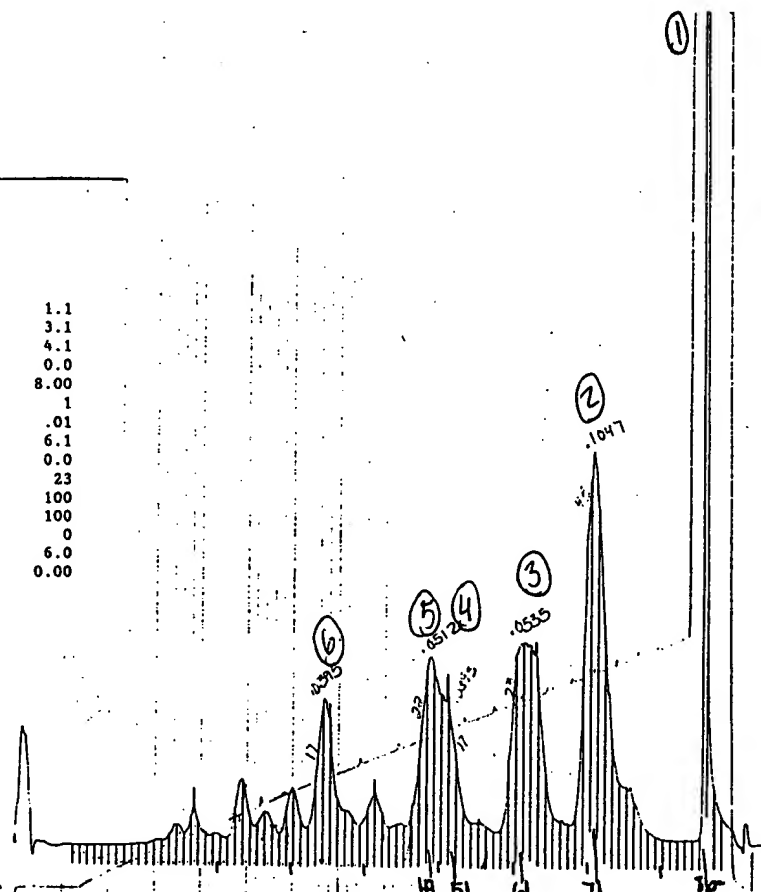
44ml SP Sephadex HP

AUFs = 1

Name = SSEPH44
Bank = 1
Number = 0

0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	VALVE.POS	4.1
0.00	CONCIB	0.0
0.00	ML/MIN	8.00
0.00	MONITOR	1
0.00	CM/ML	.01
132.0	PORT.SET	6.1
132.0	CONCIB	0.0
1454	CONCIB	23
1454	CONCIB	100
1542	CONCIB	100
1542	CONCIB	0
1542	PORT.SET	6.0
1630	CM/ML	0.00

END METHOD

To Page No. 55

Witnessed & Understood by me,

For Cees

Date

Invented by

Recorded by

Date

TITLE _____

REDACTED

Project No. 102003

Book No. 5576

55

From Page No. 54

Fractions from the six peak were concentrated and run on an SEC 3000 column #33889 from Phenomenex using method 3001.

Request # 1013

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: MF

Protein (Analyte): PEG-GCSF

Analysis Requested (RP, SEC, IEX, etc.): SEC 1280 + RI

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: MF

Method: 3001

Instrument # 2

Column: SEC 3000 #33889

Date Results Reported: _____

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	10	83-2335	-	STD	25	67	83-2347		FXN 49 1.5X
2	53	2336	.57	FXN 86 1.5X	26	49	2348		" 86 3.0X
3	16	2337	1.92	" 71 "	27	25	2349		" 71 "
4	27	2338	1.12	" 62 "	28	51	2350		" 61 "
5	39	2339	.77	" 49 "	29	31	2351		" 51 "
6	96	2340	.31	" 35 "	30	19	2352		" 49 "
7	10	2341	-	STD	31	15	2353		GCSF START
8					32	20	2354		"
9	10	53-2342	-	STD	33	60	2355		"
10	30	2343	1	GCSF (START)	34	10	2356		STD
11	43	2344	-.7023	FXN 86 3.0X	35				
12	10	2345	2.90	" 71 "	36				
13	19	2346	1.57	" 61 "	37				
14	20	2347	1.57	" 51 "	38				
15	20	2348	1.47	" 49 "	39				
16	15	2349	1	GCSF	40				
17	60	2350	1	"	41				
18	40	2351	-	STD	42				
19					43				
20	10	53-2342		STD	44				
21	30	2343		GCSF START	45				
22	60	2344		FXN 86 1.5X	46				
23	27	2345		" 71 "	47				
24	43	2346		" 62 "	48				

Notes: _____

To Page No. 56

Witnessed & Understood by me,

Date

Initiated by

Date

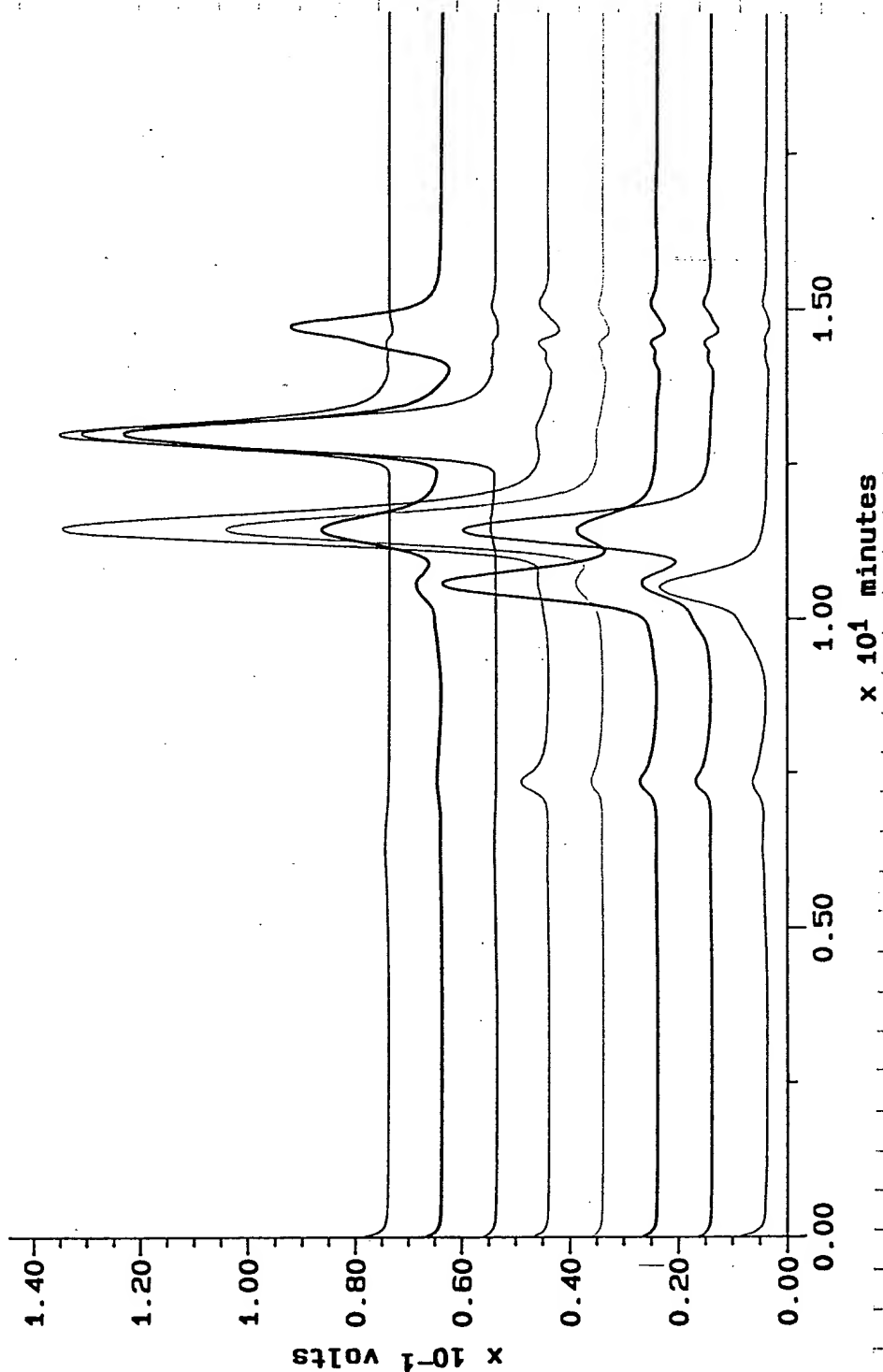
Recorded by

REDACTED

From Page No. 55

— 1.5X PEAK #3
— 1.5X PEAK #4
— 1.5X PEAK #5
— 1.5X PEAK #6

— GCSF (START)
— 1.5X PEG-GCSF
— 1.5X PEAK #1
— 1.5X PEAK #2

To Page No. 57

Witnessed & Understood by me,

Ra Elee

Date

Invented by

Christine Turner

Date

recorded by

|||

Project No. 102003

TITLE _____

REDACTED

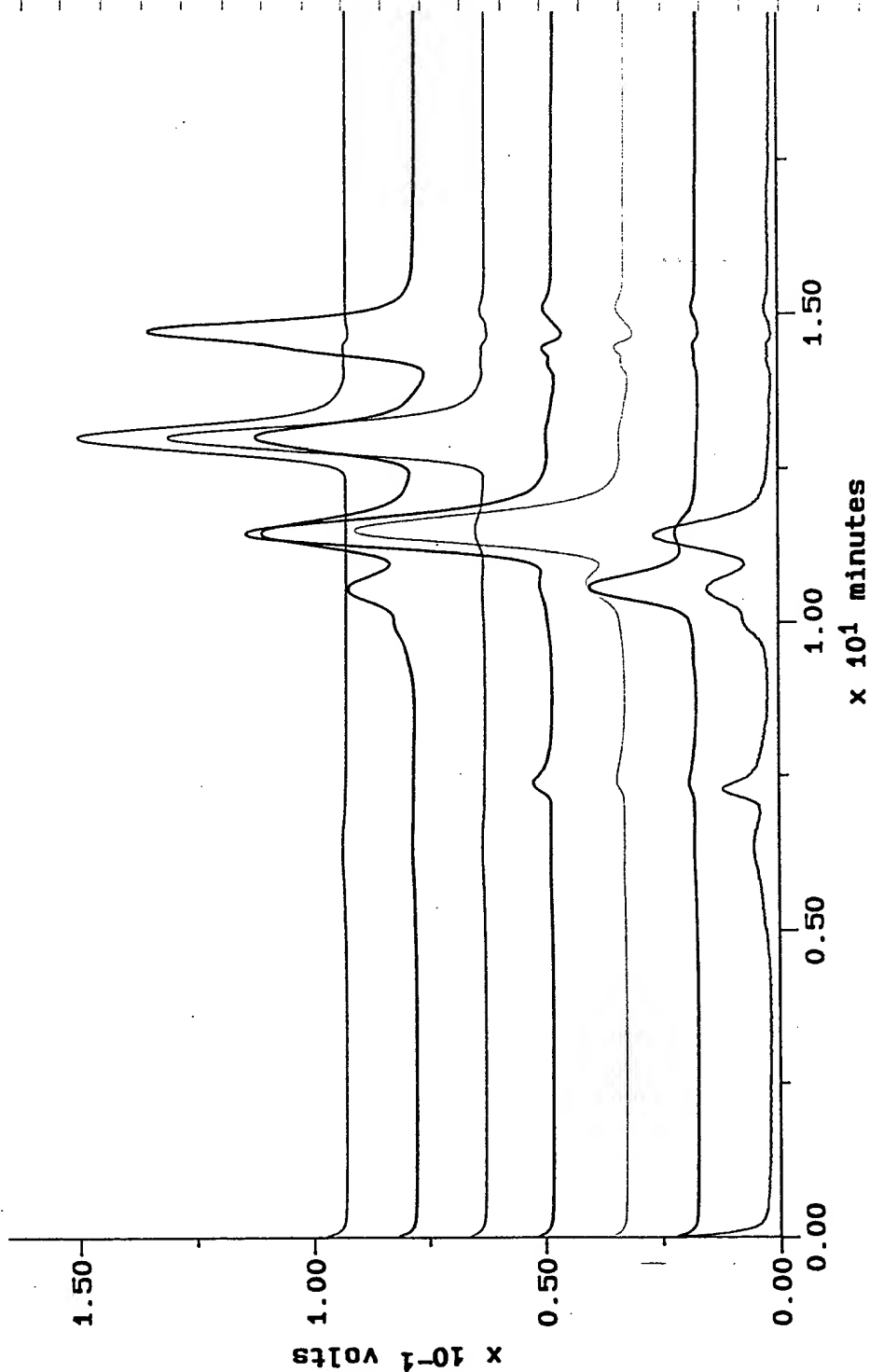
Book No. 5574

57

From Page No. 56

--- 3.0X PEAK #3
--- 3.0X PEAK #4
--- 3.0X PEAK #5

--- GCSF (START)
--- 3.0X PEG-GCSF
--- 3.0X PEAK #1
--- 3.0X PEAK #2



To Page No. 58

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Bar Weiss

Christine Jones

REDACTED

From Page No. 57

The concentrated fractions from the six peaks from each reaction mixture were also run on an SDS/PAGE Gradient Mini Gel using the SOP for Comassie stained mini gels.

GCSF-12

Date:

Operator: Chris
PEG-GCSF gel 1

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load μ L	Load μ g
MW STD	1			10.0	
GCSF (start)	2	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	0.50	0.37	16.1	8.00
PEAK #2 (bn 72)	5	1.12	0.84	11.9	10.00
PEAK #3 (bn 62)	6	0.70	0.53	19.0	10.00
PEAK #4 (bn 49)	7	0.45	0.34	29.8	10.00



GCSF-13

Date:

Operator: Chris
PEG-GCSF gel 2

NB No:

4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load μ L	Load μ g
MW STD	1			10.0	
GCSF (start)	2	1.00	0.75	4.0	3.00
PEG-GCSF 3.0X RXN	3	2.00	1.50	6.7	10.00
PEAK #1 (UNMODIFIED)	4	0.63	0.48	21.0	10.00
PEAK #2 (bn 71)	5	1.20	0.90	11.1	10.00
PEAK #3 (bn 61)	6	0.59	0.44	22.5	10.00
PEAK #4 (bn 51)	7	0.97	0.72	13.8	10.00
PEAK #5 (bn 49)	8	1.60	1.20	8.3	10.00

To Page No. 59

Witnessed & Understood by me,

Chris Oles

Date

Invented by

Signature: [Signature]

Recorded by

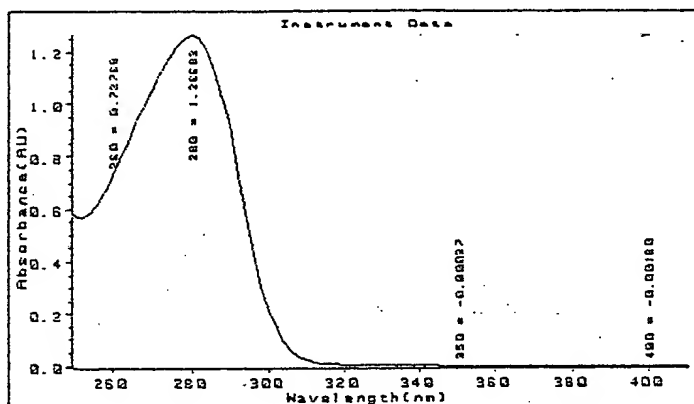
Date

TITLE _____

REDACTED

From Page No. 58

~150 ml of GCSF 5 mg/ml pH 3.25 from page 39 were placed in 8 centrifuge 10 tubes and concentrated to ~50 ml. A 100 µl aliquot was diluted 10X with WFI and the total protein concentration was calculated using A₂₈₀.



GRAPHICSCO
(LABEL / HARD COPY)

Values : L260=0.72769 L280=1.26683 L350=-0.00037 L400=-0.00180
(Stdev) : (0.00041) (0.0012) (0.00012) (0.00015)

$$\begin{aligned} & (1.26683 \text{ A}_{280} / 0.86 \text{ mg/ml}) (10 \text{ dil. factor}) = 14.7306 \text{ mg/ml} \\ & (14.7306 \text{ mg/ml}) (42.08 \text{ ml}) = 12.5 \text{ mg/ml} \times \text{ml} \\ & x = 49.59 \text{ ml} \\ & 49.59 \text{ ml} - 42.08 \text{ ml} = 7.51 \text{ ml} \end{aligned}$$

7.51 ml of WFI pH 3.25 was added to the 42.08 ml of GCSF to give 49.59 ml of 12.5 mg/ml GCSF in WFI pH 3.25.

To Page No. 60

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

From Page No. 59

The amount of 500mM Bicine to be added to the 12.5mg/ml GCSE was calculated:

$$(12.5 \text{ mg/ml})(49.59 \text{ ml}) = (10 \text{ mg/ml}) \times \text{ml}$$

$$x = 61.99 \text{ ml total}$$

$$61.99 \text{ ml total} - 49.59 \text{ ml GCSE} = 12.4 \text{ ml of 500mM Bicine}$$

12.4ml of 500mM Bicine was added to the 49.59ml of 12.5mg/ml GCSE to give 61.99ml of 10mg/ml GCSE in 100mM Bicine pH 8.0.

The amount of PEG to be combined with the GCSE to give a 1.5 Molar excess of PEG to GCSE was calculated:

$$\left(\frac{61.99 \text{ mg GCSE}}{18,800 \text{ g/mole GCSE}} \right) \left(\frac{1.5 \text{ mole PEG}}{1 \text{ mole GCSE}} \right) \left(\frac{6000 \text{ g/mole PEG}}{1} \right) = 296.76 \text{ mg PEG}$$

296.76mg of SM-MPEG was weighed into a 500ml pyrex beaker. The 61.99ml of 10mg/ml GCSE in 100mM Bicine pH 8.0 was added to the beaker and was stirred for 1 hour at room temperature. After 1 hour, the reaction mixture was diluted 5X with HEPES adjusted to a pH of 4.0 and sterile filtered into two 150ml 454 Nalgene filter units.

The 44ml 5-Sepharose column was equilibrated in 20mM NaOAc pH 4.0. The 1.5X PEG-GCSE was loaded onto the column and eluted using Method 1 from CEF. About 50mg of PEG-GCSE was loaded each time for a total of eleven runs. After each run the fractions indicated were collected and stored at 4°C.

To Page No. 61

Witnessed & Understood by me,

Frederick

Date

Invented by

Christine J. Jovan

Date

Recorded by

|||

Project No. 102003

Book No. 5574

61

TITLE

REDACTED

From Page No. 60

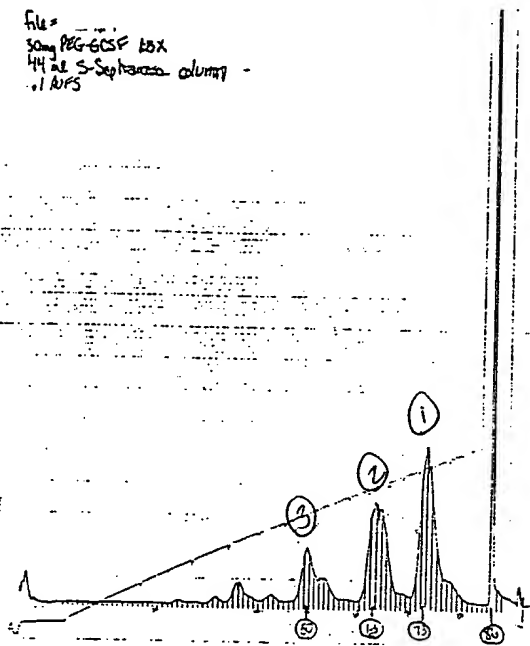
Name = SSEPH44
Bank = 1
Number = 0

File =
30mg PEG-GCSF 15X
44 ul S-Sepharose column
.1 AFS

0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	VALVE.POS	4.1
0.00	CONCIB	0.0
0.00	ML/MIN	8.00
0.00	MONITOR	1
0.00	CM/ML	.01
132.0	PORT.SET	6.1
132.0	CONCIB	0.0
1454	CONCIB	23
1454	CONCIB	100
1542	CONCIB	100
1542	CONCIB	0
1542	PORT.SET	6.0
1630	CM/ML	0.00

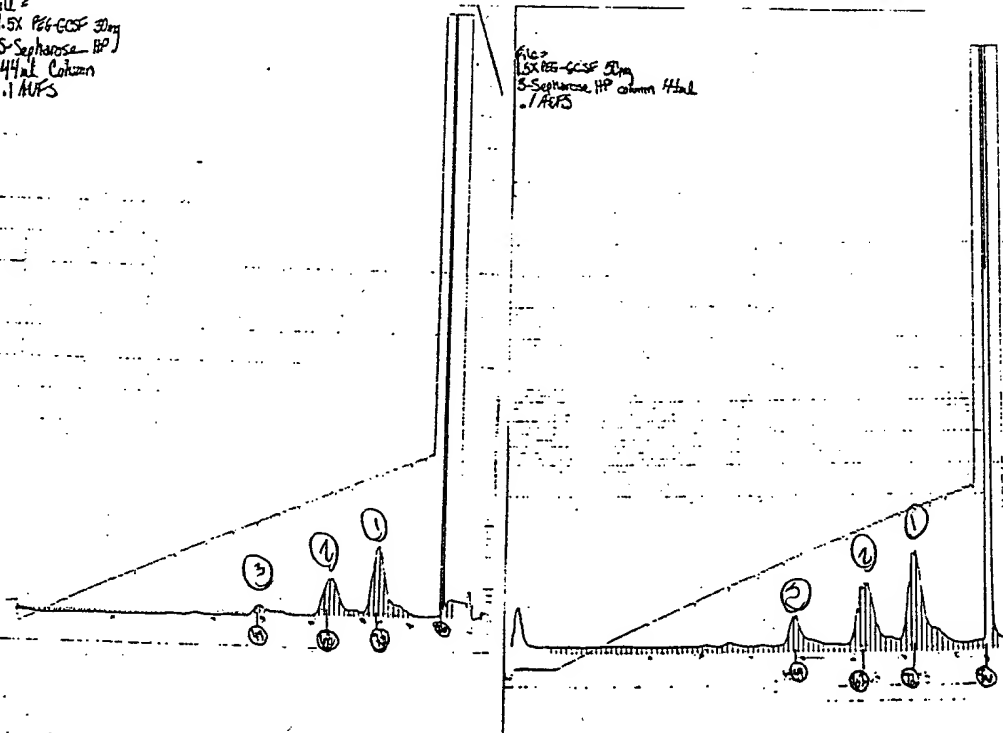
END METHOD

Fraction size = 10ml



File =
1.5X PEG-GCSF 30mg
S-Sepharose HP
44ul Column
.1 AFS

File =
1.5X PEG-GCSF 30mg
S-Sepharose HP column 44ul
.1 AFS



To Page No. 62

Witnessed & Understood by me,

[Signature]

Date

Invented by

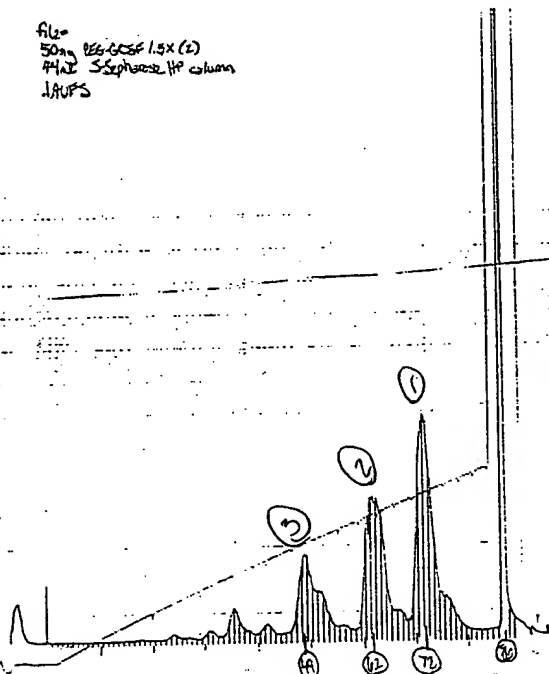
[Signature]

Date

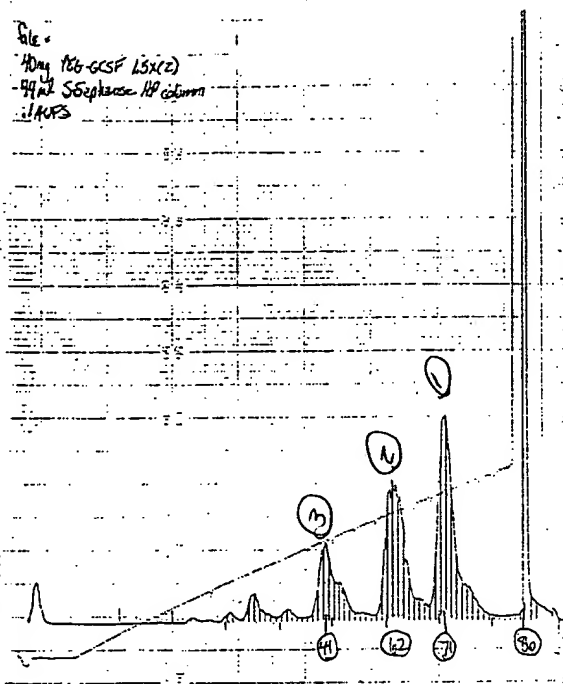
Recorded by

From Page No. 61

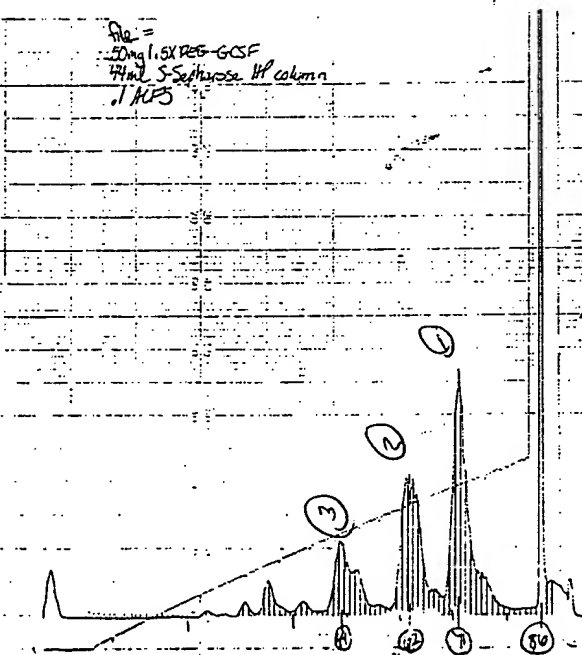
File =
50mg REG-GCSF 1.5X (2)
44ul S-Sepharose HP column
1.4KPS



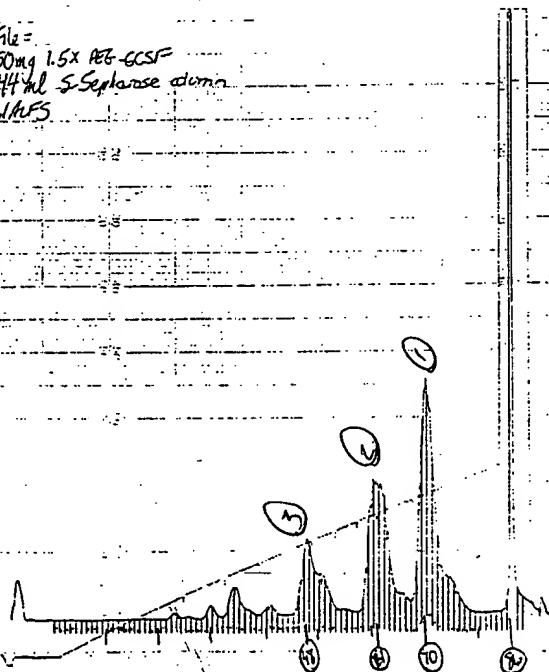
File =
40mg REG-GCSF 1.5X (2)
44ul S-Sepharose HP column
1.4KPS



File =
50mg 1.5X REG-GCSF
44ul S-Sepharose HP column
1.4KPS



File =
50mg 1.5X REG-GCSF
44ul S-Sepharose column
1.4KPS

To Page No. 63

Witnessed & Understood by me,

Date

Invented by

Date

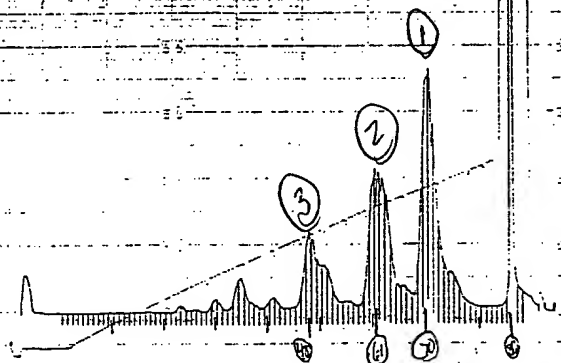
Recorded by

REDACTED

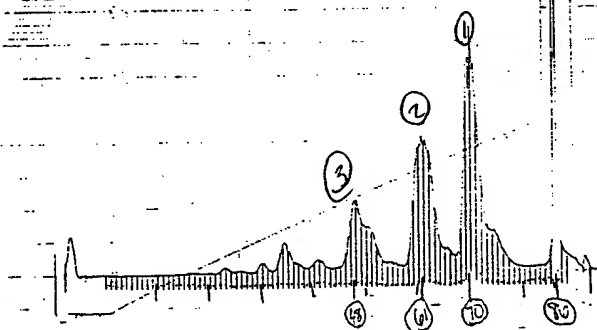
TITLE _____

From Page No. 12

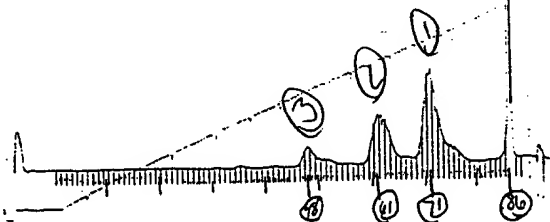
File =
50mg PEG-GCSF 1.5X
44 ml 5-Septarose HP column
1 AUFS



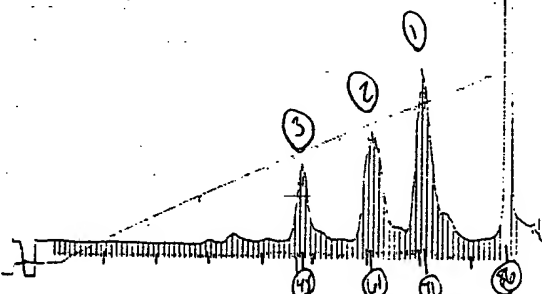
File =
50mg PEG-GCSF 1.5X
44 ml 5-Septarose HP column
1 AUFS



File =
50mg PEG-GCSF 1.5X
44 ml 5-Septarose HP column
1 AUFS



File =
50mg PEG-GCSF 1.5X
44 ml 5-Septarose HP column
1 AUFS



To Page No. _____

Witnessed & Understood by me,

Rec. [Signature]

Date

Invented by

Christine Jovan

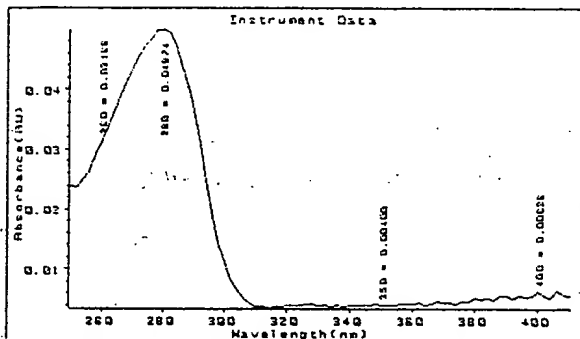
Recorded by

Date

From Page No. 63

Fractions from each of the congruent peaks of the eleven ion exchange runs were pooled and total protein concentration of the three main peaks were calculated using A_{280} :

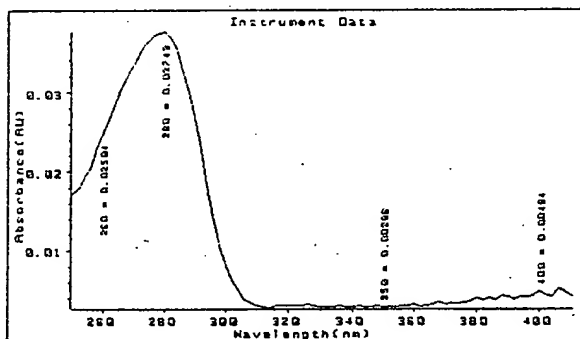
Values : L260=0.03166 L280=0.04974 L350=0.00400 L400=0.00625
(Stdev) : (0.00015) (0.00023) (0.00016) (94e-5)

PEAK #1

$$\frac{(0.04974 A_{280} - 0.004 A_{350})}{0.86 \text{ mg}} = 0.0532 \text{ mg/ml}$$

$$(0.0532 \text{ mg/ml}) (\sim 150 \text{ ml}) = \sim 8 \text{ mg}$$

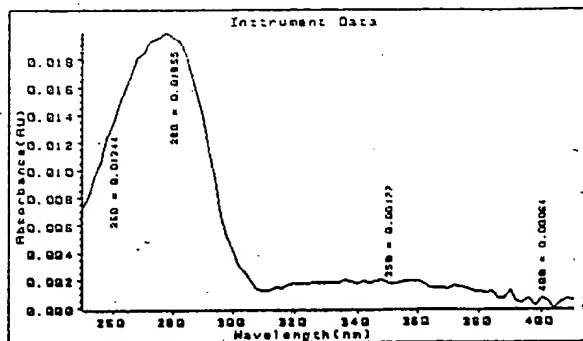
Values : L260=0.02504 L280=0.03743 L350=0.00296 L400=0.00494
(Stdev) : (0.00018) (0.00023) (0.00013) (90e-5)

PEAK #2

$$\frac{(0.03743 A_{280} - 0.00296 A_{350})}{0.86 \text{ mg}} = 0.0401 \text{ mg/ml}$$

$$(0.0401 \text{ mg/ml}) (\sim 100 \text{ ml}) = \sim 4 \text{ mg}$$

Values : L260=0.01344 L280=0.01955 L350=0.00177 L400=0.00054
(Stdev) : (0.00016) (0.00021) (0.00014) (0.00011)

PEAK #3

$$\frac{(0.01955 A_{280} - 0.00177 A_{350})}{0.86 \text{ mg}} = 0.0207 \text{ mg/ml}$$

$$(0.0207 \text{ mg/ml}) (\sim 100 \text{ ml}) = \sim 2 \text{ mg}$$

To Page No. X

Witnessed & Understood by me,

Bru Oler

Date _____

Invented by _____

Marlene Farnan

Date _____

Recorded by _____

From Page No. X

REDACTED

Materials: Pharmacia Superdex 75 HR 10/30 #9228096

MONO PEG-GCSF PEAK #1 Species (from pages 60-64)

MONO PEG-GCSF PEAK #2 Species (from pages 60-64)

MONO PEG-GCSF PEAK #3 Species (from pages 60-64)

1.0X PEG-GCSF Reaction Mixture (from page 59)

Pharmacia FPLC instrument

centriprep 10 concentrating units, Amicon

centricon 10 concentrating units, Amicon

20mM NaOAc pH 4.0 buffer

Micron 3 microconcentrators, Amicon

I.S.S. 4-20% Gradient Mini Gels

Comassie SDP solutions

Waters HPLC instrument

Phenomenex SEC 3000 column #33889 #41930

100mM NaPhos pH 6.9 in MilliQ water

To Page No. 66

Witnessed & Understood by me,

Paul Lee

Date

Invented by

2

Christine Johnson

Recorded by

Date

From Page No. 15

REDACTED

Procedure:

The Superdex 75 column was set up on the FPLC and equilibrated in 20mM NaOAc pH 4.0. 2 mg of the 1.5X PEG-GCZF reaction mixture was loaded onto a 1 ml loop and the eluted through the column using Method 1 from CEF₂ (see Fig. 1)

The fractions from peak #1 of the Mono pegylated species were concentrated in centrprep 10 tubes to about 4 ml at 2 mg/ml. 1 ml of this peak #1 solution was loaded onto the column and eluted using Method 1 of CEF. Fractions 23 and 24 were collected, pooled, and stored at 4°C. (See Fig. 2)

Fig. 1

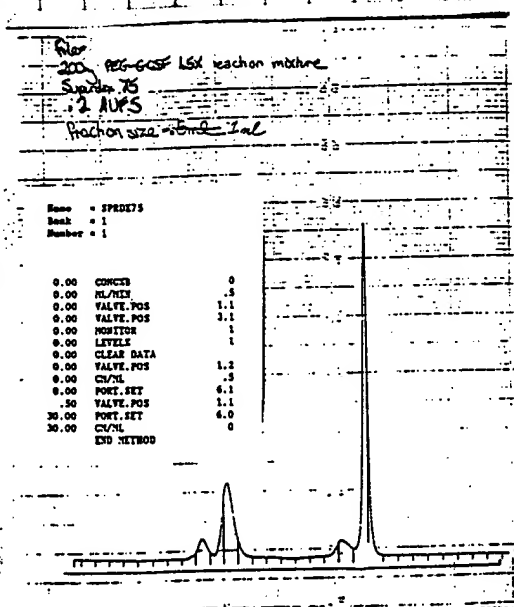
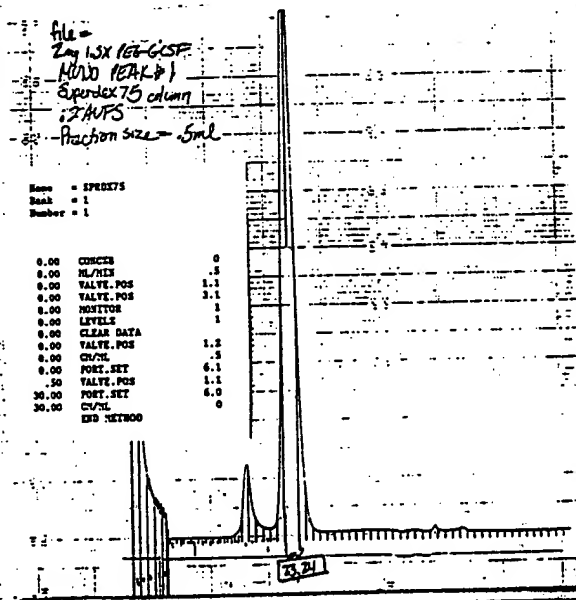


Fig. 2



To Page No. 17

Witnessed & Understood by me,

Gre Oer

Date

Invented by

Martine Jann

Recorded by

Date

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TITLE

REDACTED

From Page No. 66

The fractions from peak #2 of the MONO PEG species were concentrated in centrifuge 10 tubes to about 4 ml at 1 mg/ml. 1 ml of this peak #2 solution was loaded onto the column and eluted using Method 1 from CEF. Fractions 23 and 24 were collected, pooled, and stored at 4°C. (See Fig. 1)

Another ml was loaded onto the column and eluted using Method 1 from CEF. Fractions 23 and 24^{and 25} were collected and pooled with the fractions from , and then stored at 4°C. (see Fig. 2)

fig. 1

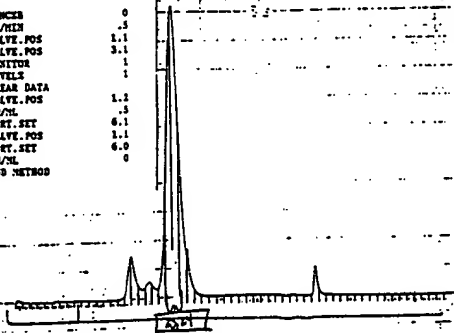
fig. 2

File =
1 mg 1.5X PEG-GCSE
MONO PEAK #2
Sperdex 75 column
2 AFS
Fraction size = .5 ml

Name = SPDEX75
Bank = 1
Number = 1

0.00 CONCENT
0.00 NL/ML
0.00 VALVE.POS
0.00 VALVE.POS
0.00 MONITOR
0.00 LEVELS
0.00 CLEAR DATA
0.00 VALVE.POS
0.00 CUV/ML
0.00 PORT.SET
0.00 VALVE.POS
30.00 PORT.SET
30.00 CUV/ML
END METHOD

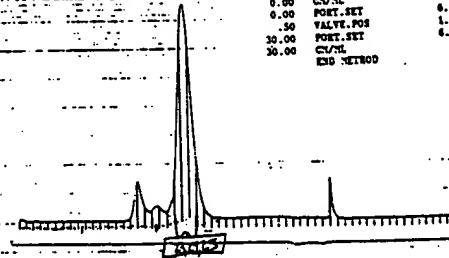
0
.5
1.1
3.1
1
1
1.2
.5
6.1
1.1
6.0
0



File =
1 mg 1.5X PEG-GCSE
MONO PEAK #2
Sperdex 75 column
2 AFS
Fraction size = .5 ml

Name = SPDEX75
Bank = 1
Number = 1

0.00 CONCENT
0.00 NL/ML
0.00 VALVE.POS
0.00 VALVE.POS
0.00 MONITOR
0.00 LEVELS
0.00 CLEAR DATA
0.00 VALVE.POS
0.00 CUV/ML
0.00 PORT.SET
0.00 VALVE.POS
30.00 PORT.SET
30.00 CUV/ML
END METHOD



To Page No. 68

Witnessed & Understood by me,

Butler

Date

Invented by

Christine J. Juran

Date

Recorded by

from Page No. 17

The fractions from peak #3 of the Mono pegylated species were concentrated in centrifuge 10 tubes to about 25ml at 0.5 ml. 1 ml of this peak #3 solution was loaded onto the column and eluted using method 1 from CEF. Fractions 23 and 24 were collected, pooled, and stored at 4°C. (see fig. 1)

Another ml of the remaining peak #3 solution was concentrated down to 1ml using centrifuge tube and was loaded onto the column and eluted using method 1 from CEF. Fractions 23 and 24 were collected, pooled with the fractions from 110292-1, and stored at 4°C. (see fig. 2)

fig. 1

File #
102003
2mg 15K PEG-GCSP
H2O00 PEAK #3
Superdex 75 column
2.0 ml
Fraction size = 0.5 ml

Name = SPED75
Batch = 1
Number = 1

0.00	CORRECTION	0
0.00	HL/HIS	0.5
0.00	VALVE.POS	1.1
0.00	VALVE.POS	1.1
0.00	MONITOR	1
0.00	LEVELS	1
0.00	CLEAR DATA	1.2
0.00	VALVE.POS	1.1
0.00	C/V/L	0.5
0.00	PORT.SET	6.1
0.00	VALVE.POS	1.1
0.00	PORT.SET	6.1
0.00	C/V/L	0.5
0.00	END NETROD	0

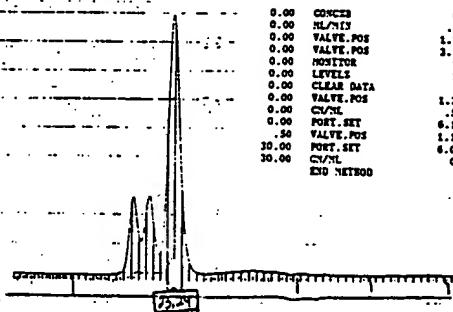


fig. 2

File #
102003
2mg 15K PEG-GCSP
H2O00 PEAK #3
Superdex 75 column
2.0 ml
Fraction size = 0.5 ml

Name = SPED75
Batch = 1
Number = 1

0.00	CORRECTION	0
0.00	HL/HIS	0.5
0.00	VALVE.POS	1.1
0.00	VALVE.POS	1.1
0.00	MONITOR	1
0.00	LEVELS	1
0.00	CLEAR DATA	1.2
0.00	VALVE.POS	1.1
0.00	C/V/L	0.5
0.00	PORT.SET	6.1
0.00	VALVE.POS	1.1
0.00	PORT.SET	6.1
0.00	C/V/L	0.5
0.00	END NETROD	0

To Page No. 69

Witnessed & Understood by me,

Er Qee

Date

Invented by

Christine Jones

Recorded by

Date

REDACTED

One ml of the peak #1 solution was loaded onto the column and eluted using method 1 from CEF. Fractions 23 and 24 were collected, pooled with the fractions from , and stored at 4°C. (see fig. 2)

fig. 1

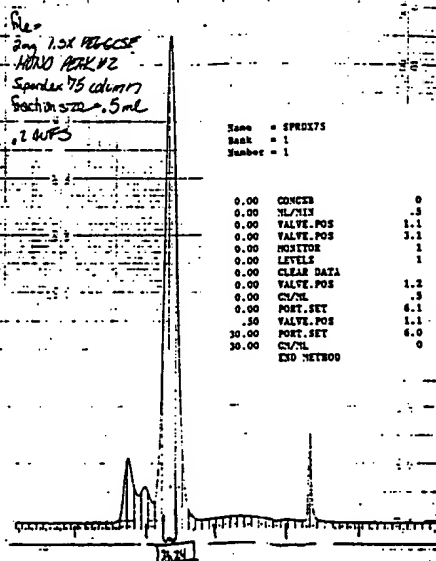
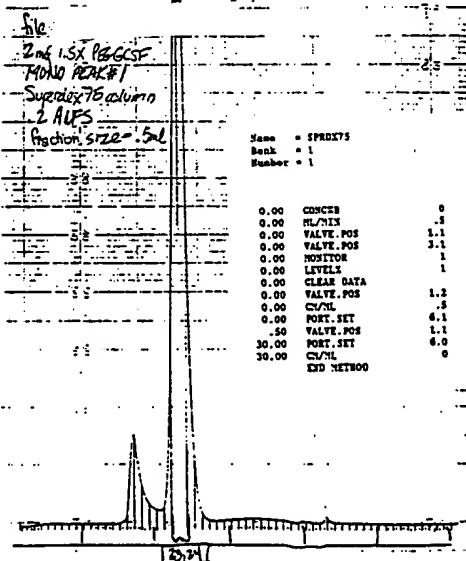


fig. 2



To Page No. 70

Witnessed & Understood by me,

Date

Invented by

Date**Recorded by**

TITLE _____

REDACTED

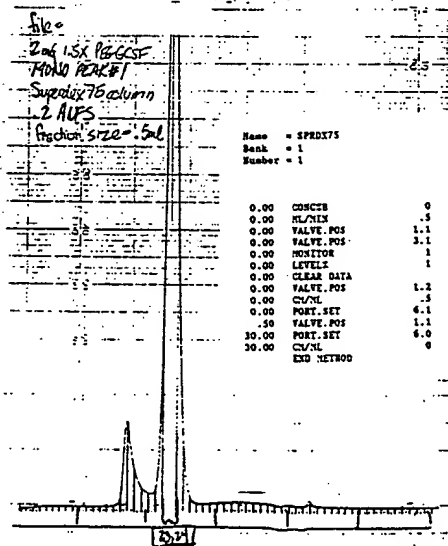
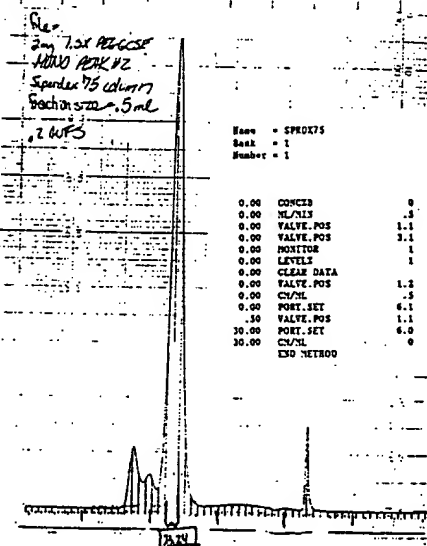
From Page No. 66

The remaining peak #2 solution was concentrated down to 1ml using centrifuge tube and was loaded onto the column and eluted using method 1 from CEF. Fractions 23 and 24 were collected, pooled with the fractions from _____ and _____, and stored at 4°C. (see Fig. 1)

One ml of the peak #1 solution was loaded onto the column and eluted using method 1 from CEF. Fractions 23 and 24 were collected, pooled with the fractions from _____, and stored at 4°C. (see Fig. 2)

Fig. 1

Fig. 2



To Page No. 70

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 109

One ml of the peak #1 solution was loaded onto the column and was eluted using Method 1 from CEF. Fractions 23 and 24 were collected and pooled with the fractions from 10302-1 and 110492-2 and stored at 4°C. (see fig. 1)

Another ml of the peak #1 was loaded onto the column and was eluted using Method 1 from CEF. Fractions 23 and 24 were collected and pooled with the fractions from _____, and _____ and stored at 4°C. (see fig. 2)

fig. 1

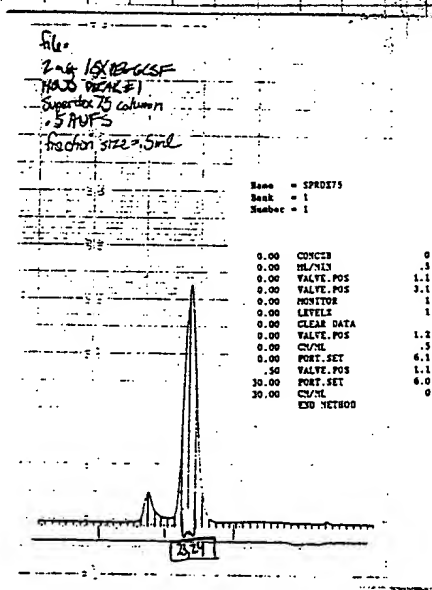
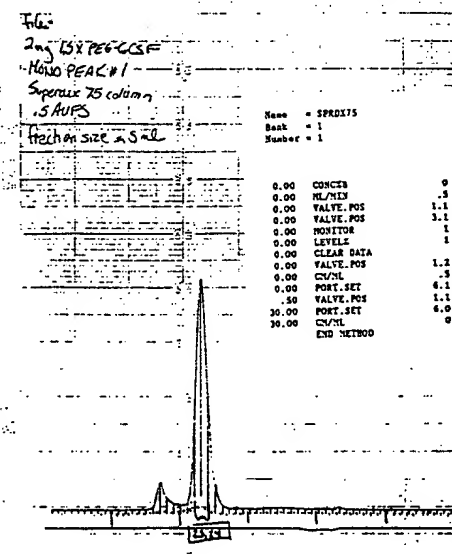


fig. 2



To Page No. _____

Witnessed & Understood by me,

B. C. Lee

Date

Invented by

Christine Farnan

Recorded by

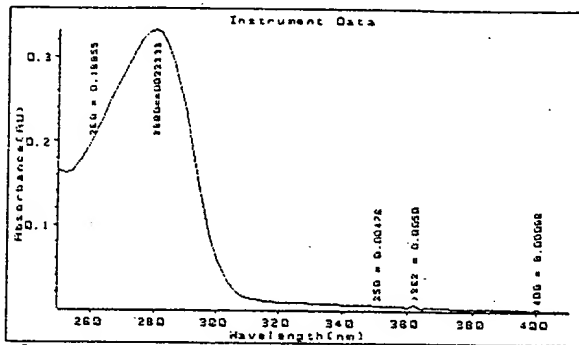
Date

TITLE _____

REDACTED

From Page No. 10

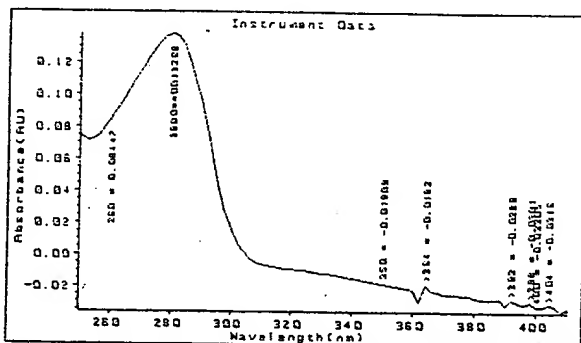
An aliquot of 150 μ l was taken from each of the three peak solutions and total protein concentration of the mono pegylated species was calculated using A_{280} :



Values : L260=0.1855 L280=0.33131 L350=0.00476 L400=0.0008
(Stdev) : (0.00021) (0.00032) (0.00015) (0.00018)

PEAK #1

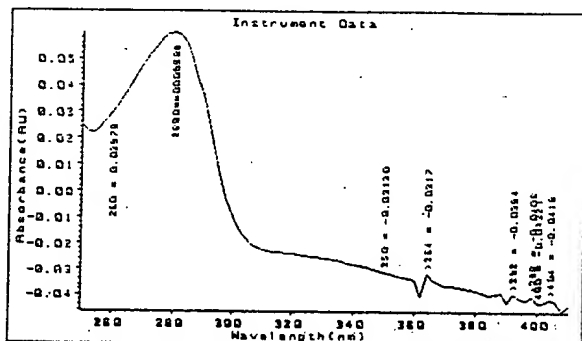
$$\begin{aligned} & (.33131^{A_{280}} - 0.00476^{A_{350}}) / .86 = .3797 \text{ mg/ml} \\ & (.3797 \text{ mg/ml}) (\sim 4 \text{ ml}) = \sim 1.5 \text{ mg} \end{aligned}$$



Values : L260=0.0417 L280=0.13768 L350=-0.01909 L400=-0.03305
(Stdev) : (0.00018) (0.00030) (0.00019) (0.00016)

PEAK #2

$$\begin{aligned} & (.13768^{A_{280}}) / .86 = .1601 \text{ mg/ml} \\ & (.1601 \text{ mg/ml}) (\sim 3 \text{ ml}) = \sim .5 \text{ mg} \end{aligned}$$



Values : L260=0.0178 L280=0.05984 L350=-0.03130 L400=-0.04321
(Stdev) : (0.00020) (0.00027) (0.00016) (0.00020)

PEAK #3

$$\begin{aligned} & (.05984^{A_{280}}) / .86 = .0696 \text{ mg/ml} \\ & (.0696 \text{ mg/ml}) (\sim 2 \text{ ml}) = \sim .1 \text{ mg} \end{aligned}$$

To Page No. 12

Witnessed & Understood by me,

Pro Lee

Date

Invented by

Christine Furrer

Date

Recorded by

From Page No. 71

REDACTED

Aliquots from the three peaks were concentrated in 500ul Micron microconcentrators from Amicon and run on 50S/PAGEZ Gradient Mini Gel using the SOP for Comassie stained mini gels:

$$\begin{aligned} \text{Peak \#1} - (500\text{ul}) (0.5797\text{mg/ml}) &= x\text{mg/ml} (270\text{ul}) & x &= .7031\text{mg/ml} \\ \text{Peak \#2} - (500\text{ul}) (0.1601\text{mg/ml}) &= x\text{mg/ml} (90\text{ul}) & x &= .8894\text{mg/ml} \\ \text{Peak \#3} - (500\text{ul}) (0.0696\text{mg/ml}) &= x\text{mg/ml} (10\text{ul}) & x &= 5.48\text{mg/ml} \end{aligned}$$

GCSF-14

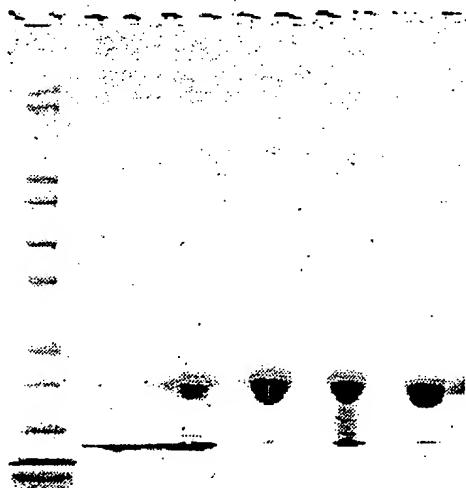
Date:
Operator: Chris
PEG-GCSF gel 1

NB No.: 5576-39
4-20% Gradient Mini Gel

Running Conditions

constant current: 25 mA
all non-reduced
Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc mg/ml	Load uL	Load ug
MW STD	1			10.0	
GCSF (start)	3	1.00	0.75	4.0	3.00
PEG-GCSF 1.5X RXN	5	2.00	1.50	6.7	10.00
PEAK #1	7	0.70	0.53	19.0	10.00
PEAK #2	9	0.89	0.67	15.0	10.00
PEAK #3	11	3.48	2.61	3.8	10.00

To Page No. 73

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

From Page No. 72

Another aliquot from peak #3 was concentrated in a Micron concentrator and all three peaks (concentrated) were run on an SEC3000 column #41930 from Phenomenex.

Request # 1056

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: <u>C.F.</u>
Protein (Analyte): <u>MPEG-GCSE MONO Polyated Species</u>
Analysis Requested (RP, SEC, IEX, etc.): <u>SEC</u>
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CF
 Method: 3007
 Instrument # 2

Column: SEC 3000 # 41930
 Date Results Reported: _____

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	10	53-2425	1	STD	25				
2	15	53-2426	2	GCSE (start)	26				
3	(15)	53-2427	2	Rxn Mix 1.5X	27				
4	(15)	53-2428	.7	Peak #1	28				
5	(15)	53-2429	.8	Peak #2	29				
6	(15)	53-2430	.35	Peak #3	30				
7	(15)	53-2431	2	GCSE (start)	31				
8	10	53-2432	1	STD	32				
9					33				
10					34				
11					35				
12					36				
13					37				
14					38				
15					39				
16					40				
17					41				
18					42				
19					43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 74

Witnessed & Understood by me,

[Signature]

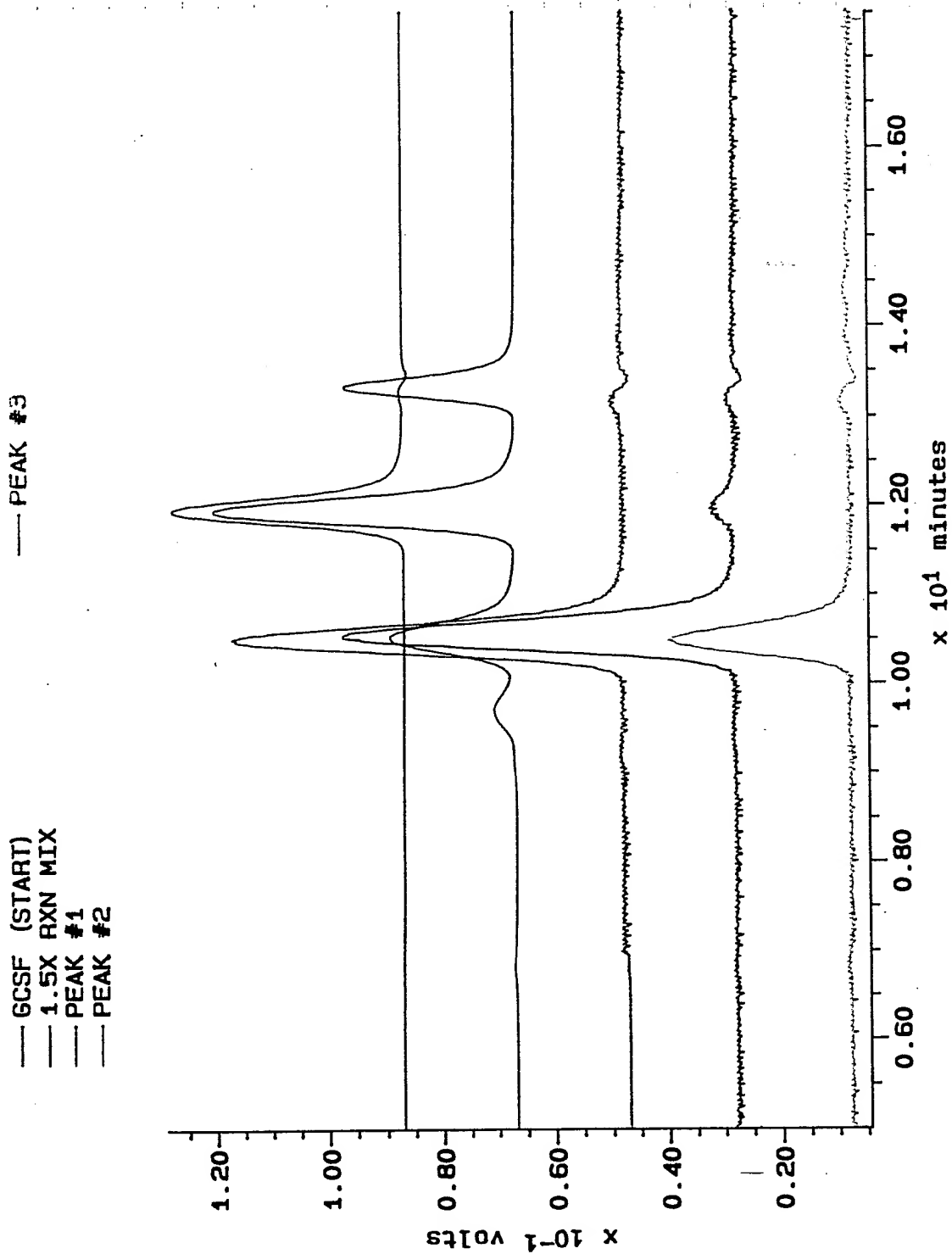
Date

Invented by

[Signature]

Date

Recorded by

From Page No 73To Page No. X

Witnessed & Understood by me,

[Signature]

Date

Invented by

[Signature]

Recorded by

Date

From Page No. ~~4~~

REDACTED

400ul (200ul in each endpoint) of each peak or species from page 71 was aliquoted along w/ 200ul of the buffer and 200ul of the starting material from page 39. This material was then given to Wendy Brenner in Analytical Services for in vitro bioactivity assay.

PLEASE SUBMIT AT LEAST 200 μ L OF SAMPLE. ASSAYS ARE DONE ON TUESDAYS; PLEASE CALL AHEAD AND SUBMIT YOUR SAMPLES BY 1200PM ON MON IF YOU WISH THEM TO GET IN THAT TUESDAY. RESULTS WILL BE IN THE MAIL BY THE NEXT MONDAY EVENING.

DATE:

EXTENSION: 2241

NAME Chris Felter

MAILBOX: 8-1-A-215

DO YOU WISH TO GET YOUR SAMPLES BACK? (YES OR NO)

[illegible]

not enough sample

[illegible]

SPECIAL INSTRUCTIONS:

IF YOU HAVE ANY COMMENTS OR QUESTIONS PLEASE CONTACT WENDY (x2365) OR LISA (x4059)

To Page No. 76

Witnessed & Understood by me,

Date _____

Invented by

Date

Recorded by

Project No. 102003Book No. 5516

TITLE _____

REDACTED

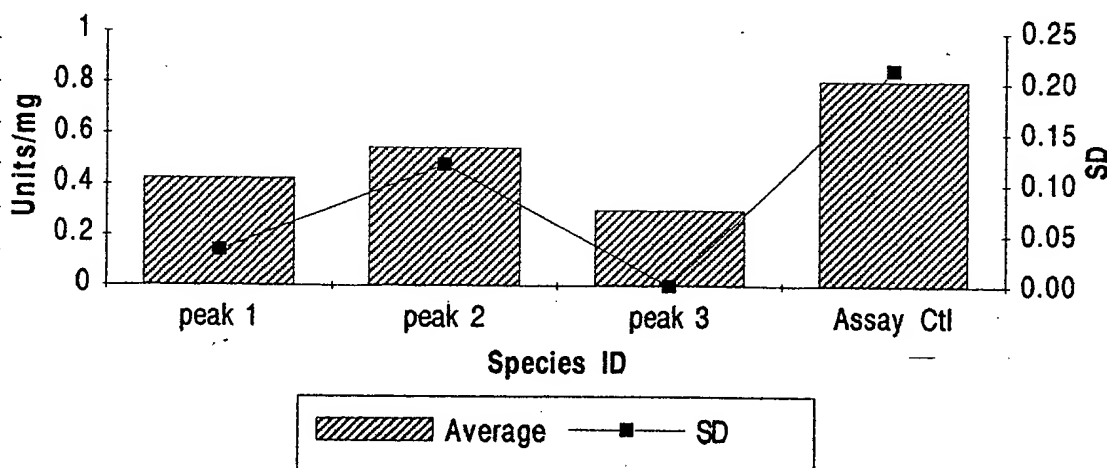
From Page No. 15

SUMMARY OF G-CSF BIOASSAY #397

SUMMARY DATE Standard curve format					
SAMPLE	INITIAL DILUTION	mg/mL from A-280	U/mL assayed x E+08	U/mg calculated x E+08	
1 630EOC S0142 CONTROL	500000	0.29	0.19	0.64	
2 6101 S0140 PREFORM	5000000	2.93	1.61	0.55	
3 6102 S0140 PREFORM	5000000	3.02	1.61	0.60	
4 BLANK	-	-	-	-	
5 PEAK 1 RUN 1	500000	0.3852	0.15	0.40	
6 PEAK 2 RUN 1	100000	0.1601	0.10	0.63	
7 PEAK 3 RUN 1	100000	0.0696	0.02	0.30	
8 PEAK 1 RUN 2	500000	0.3852	0.17	0.45	
9 630EOC S0142 CONTROL	500000	0.29	0.21	0.74	
10 PEAK 2 RUN 2	100000	0.1601	0.07	0.46	
11 PEAK 3 RUN 2	100000	0.0696	0.02	0.30	
12 GCSF CONTROL	5000000	5	3.31	0.66	
13 E. coli LOT G204	500000	0.509	0.40	0.78	
14 CHO PRODUCT LOT 3	1000000	1.088	1.48	1.36	
15 CHO PRODUCT LOT 4	1000000	1.001	1.31	1.31	
16 FILGRASTIM STD LOT G1115	500000	0.503	1.16	2.31	
17 CHUGAI G-CSF RUN1	500000	0.25	0.41	1.62	
18 CHUGAI G-CSF RUN 2	500000	0.25	0.32	1.27	
19 692L2 S0347	500000	0.20	0.19	0.66	
20 630EOC S0142 CONTROL	500000	0.29	0.30	1.05	
630EOC AVERAGE OF 3 = 0.81 X E+08 U/MG					
U = NBSC STANDARDIZED UNITS					

	Run1	Run2	Run3	Average	SD	% of Starting G
peak 1	0.40	0.45		0.425	0.04	0.64
peak 2	0.63	0.46		0.545	0.12	0.83
peak 3	0.30	0.30		0.30	0.00	0.45
630EOC S0142 control	0.64	0.74	1.05	Assay Ctl	0.81	1.23
our GCSF	0.66					

Mono-PEG-GCSF Species Bioassay Results

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. X

REDACTED

AMGEN

PROTEIN STRUCTURE

MEMORANDUM

To: Elise Gabriel and Chris Farrar

From: Chris Clogston and Hsieng Lu *HL*

Date:

Subj: N-terminal Sequence Analysis of Monopegylated GCSF Peaks 1-3.

N-terminal sequence analysis was performed on samples submitted November 9, 1992. One nmol (based on provided concentrations) of each sample was loaded on an Applied Biosystems 477A protein sequencer and run for forty-three cycles according to analytical method A0102. Peak 3, containing 680 pmols only, was loaded in its entirety. Recovery tables and repetitive yield graphs are included for each sample.

Peak 1 is almost entirely N-terminally blocked. If provided concentration is correct, initial yield for this sample is approximately 1%. Lys¹⁷ is recovered at somewhat low levels but well within the scatter of the data. The other three lysines were not recovered in this analysis, however, this is probably a combination of the extremely low initial recovery and pegylation at these sites. Sequencing forty-two residues on approximately 15 pmol of sequenceable material is taking instrument performance to an extreme, therefore, the analysis is only qualitative.

Peak 2 is partially N-terminally blocked as initial yield is well below 50%. All lysine recoveries appear normal based on their positions on the repetitive yield regression line.

Peak 3 is probably partially N-terminally blocked as GCSF, in lot release analyses, always has an initial yield recovery of greater than 60%. Of the lysine recoveries, Lys¹⁷, Lys²⁴, and Lys³⁵ all are recovered normally, based on their positions on the repetitive yield regression line. Only Lys⁴¹ is not recovered, indicating probable pegylation at this site.

The data indicates that these species are either not monopegylated or are mixtures of monopegylated species. In each case, the N-terminus is at least partially modified. The extent of N-terminus modification can only be determined with accurate sample protein concentrations. Peptide analysis is required to precisely identify the sites and extent of pegylation.

To Page No. 78

Witnessed & Understood by me,

Chris Clogston

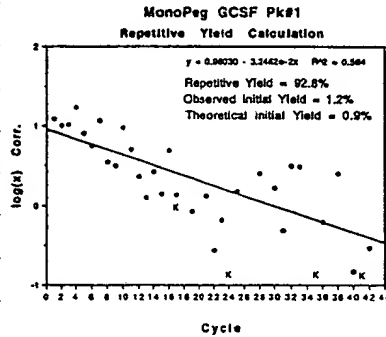
Date

Invented by

Date

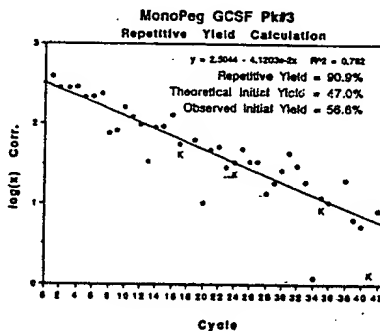
Recorded by

Christine Johnson

From Page No. 11

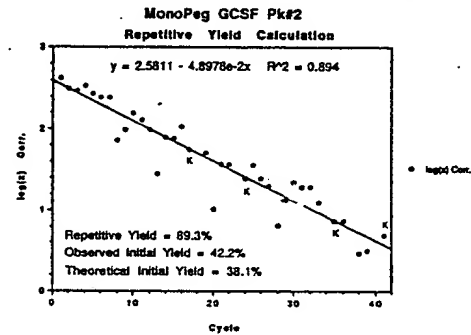
MonoPeg GCSF Pk#2 Data

Cycle	Cycle	Prod. Recov.	Background	Corr. Prod.	log(x) Corr.
1	1	422.27	0.00	422.27	2.626
2	2	320.26	12.81	307.47	2.486
3	3	298.32	3.00	295.32	2.470
4	4	348.92	16.84	332.08	2.520
5	5	277.42	9.54	267.88	2.428
6	6	273.09	33.00	240.09	2.380
7	7	249.65	9.88	239.77	2.380
8	8	82.84	8.28	74.56	1.861
9	9	106.04	8.28	97.76	1.890
10	10	212.50	33.00	179.50	2.197
11	11	148.47	19.65	128.82	2.111
12	12	132.58	34.92	97.66	1.990
13	13	37.43	6.84	30.59	1.484
14	14	83.49	15.13	68.36	1.834
15	15	144.32	66.29	78.03	1.889
16	16	173.58	46.99	126.59	2.028
17	17	63.81	8.93	54.88	1.740
18	18	119.72	46.72	73.00	1.706
19	19	28.21	16.00	12.21	1.009
20	20	78.44	36.88	41.56	1.543
21	21	50.57	14.31	36.26	1.559
22	22	33.21	8.81	24.40	1.387
23	23	47.85	11.67	36.18	1.556
24	24	60.16	35.73	24.43	1.388
25	25	43.82	26.51	17.31	1.238
26	26	14.38	8.04	6.34	0.801
27	27	44.18	32.69	11.49	1.130
28	28	55.84	34.01	21.83	1.335
29	29	53.28	34.01	19.27	1.283
30	30	75.43	56.31	19.12	1.281
31	31	42.60	30.32	12.28	1.089
32	32	10.80	12.13	-1.33	NAN(036)
33	33	12.86	5.44	7.42	0.870
34	34	64.38	57.06	7.32	0.863
35	35	32.10	29.20	2.90	0.462
36	36	9.84	6.80	3.04	0.497
37	37	10.11	5.29	4.82	0.684
38	38	52.24	56.91	-3.67	NAN(036)



MonoPeg GCSF Pk#1 Data

Cycle	Cycle	Prod. Recov.	Background	Corr. Prod.	log(x) Corr.
1	1	12.30	0.00	12.30	1.090
2	2	11.72	1.77	9.95	0.996
3	3	12.05	2.81	9.24	1.010
4	4	28.86	9.48	19.38	1.240
5	5	14.86	8.94	5.92	0.904
6	6	16.18	10.68	5.50	0.747
7	7	16.80	5.28	11.52	1.068
8	8	12.22	6.75	5.47	0.540
9	9	11.89	6.75	5.14	0.497
10	10	48.70	20.24	28.46	1.223
11	11	23.17	16.10	7.07	0.705
12	12	33.27	31.04	2.23	0.344
13	13	13.27	12.04	1.23	0.090
14	14	15.13	12.46	2.67	0.427
15	15	62.16	60.78	1.40	0.146
16	16	48.40	60.78	-1.40	0.683
17	17	6.28	6.92	-0.64	0.130
18	18	66.84	66.01	0.83	-0.061
19	19	15.65	16.07	-0.42	NAN(036)
20	20	34.37	32.25	2.12	0.121
21	21	13.30	13.03	0.27	-0.369
22	22	1.84	1.00	0.84	-0.180
23	23	7.05	6.10	0.95	NAN(036)
24	24	10.62	8.11	2.51	0.179
25	25	34.33	34.59	-0.26	NAN(036)
26	26	20.31	23.83	-3.52	NAN(036)
27	27	6.91	6.42	0.49	0.396
28	28	24.04	28.73	-4.69	NAN(036)
29	29	31.64	31.16	0.48	0.320
30	30	59.34	56.24	3.10	0.481
31	31	33.63	30.61	3.02	0.460
32	32	13.80	14.08	-0.28	NAN(036)
33	33	6.35	6.56	-0.21	NAN(036)
34	34	54.06	53.45	0.61	-0.215
35	35	33.73	31.27	2.46	0.391
36	36	5.28	5.87	-0.59	NAN(036)
37	37	5.17	5.02	0.15	-0.824
38	38	5.58	5.58	-0.02	NAN(036)
39	39	53.70	53.41	0.29	-0.538



MonoPeg GCSF Pk#3 Data

Cycle	Cycle	Prod. Recov.	Background	Corr. Prod.	log(x) Corr.
1	1	385.14	0.00	385.14	2.586
2	2	301.22	20.85	280.37	2.444
3	3	282.41	4.33	278.08	2.444
4	4	308.83	24.00	284.83	2.455
5	5	226.93	15.55	211.38	2.331
6	6	247.29	30.65	216.64	2.336
7	7	248.66	14.89	233.77	2.369
8	8	92.44	6.92	85.52	1.878
9	9	89.64	6.92	82.72	1.916
10	10	215.89	54.74	161.15	2.207
11	11	141.87	20.43	121.44	2.084
12	12	131.43	32.28	99.15	1.992
13	13	46.90	12.66	34.24	1.531
14	14	106.15	17.82	88.33	1.936
15	15	171.00	79.41	91.59	1.962
16	16	206.17	79.41	126.76	2.113
17	17	60.28	4.35	55.93	1.748
18	18	187.12	105.55	81.57	1.788
19	19	29.31	19.24	10.07	1.012
20	20	89.03	42.04	46.99	1.672
21	21	70.85	16.90	53.95	1.714
22	22	43.13	14.80	28.33	1.455
23	23	37.84	5.12	32.72	1.515
24	24	66.36	17.93	48.43	1.688
25	25	77.13	43.53	33.60	1.528
26	26	66.28	31.51	34.76	1.529
27	27	18.54	5.82	12.72	1.127
28	28	96.77	48.24	48.53	1.681
29	29	71.72	44.85	26.87	1.428
30	30	88.08	44.85	43.23	1.641
31	31	102.15	72.17	30.00	1.477
32	32	36.88	39.90	-13.02	NAN(036)
33	33	16.38	17.19	-0.81	0.279
34	34	15.44	3.46	11.98	1.076
35	35	81.43	76.73	4.70	1.029
36	36	67.60	37.43	30.17	1.308
37	37	16.02	9.61	6.41	0.807
38	38	11.68	6.38	5.30	0.734
39	39	2.80	2.36	-0.44	NAN(036)
40	40	47.48	58.34	-10.86	0.811

To Page No. 12

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. X

REDACTED

Materials: GCSF lot #T6702 (~1200mg)
 SCH-M PEG lot #84-7 UCC (~700mg)
 350ml Amicon stirred cell
 YM10 46mm membrane (x5)
 1 liter .2u Costar Filter unit
 1 liter .45u Nalgene filter unit
 Pharmacia XK 50/130 column
 Pharmacia S-Sepharose FF lot #03673 resin (~700ml)
 4L Beakers (x2)
 Cole-Parmer Masterflex Quick Load Peristaltic pump
 Pharmacia Biosep SEC 3000 column #41930
 Waters HPLC system
 I.S.S H-2076 Gradient Mini Gel
 WFI (Water for Injection) Baxter pH 3.25
 500mM Bicine in WFI pH 8.0
 .5 M NaCl in WFI
 .1 M NaOH in WFI
 500mM NaOAc in WFI pH 4.00
 20 mM NaOAc in WFI pH 4.0 (16L)
 20mM NaOAc, 1M NaCl in WFI pH 4.0 (2L)
 100mM NaPhos in Milli Q pH 6.9
 Comassie 20P solutions

To Page No. 80

Witnessed & Understood by me,

Pr. Lee

Date

Invented by

Michael J. Jones

Date

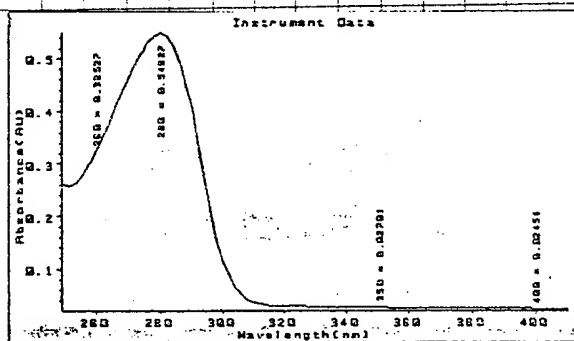
Recorded by

From Page No. 10

REDACTED

Procedure

~350ml of GCSF lot #S0140Tle702 was placed in an Amicon stirred cell and concentrated to ~200ml. Another 150ml of GCSF was added to the cell and concentrated to ~100ml. The GCSF was then buffer exchanged into WFI pH 3.25 via a pressurized reservoir connected to the stir cell. ~700ml (4 retentate volumes) were exchanged. A 50ul aliquot was diluted 20X with WFI and its protein concentration was calculated using UV_{280} .



```
GRAPH/CSO  
LABEL / HARD COPY
```

	0	1	2	3	4	5	6	7	8	9	E
Values :	L260=0.32527	L280=0.54927	L350=0.02701	L400=0.0245							
(Stdev) :	(0.00018)	(0.00047)	(0.00015)	(0.00015)							

$$(.54927 - .02701) / .86 = .6073 \text{ mg/ml} \times 20 = 12.15 \text{ mg/ml}$$

$$(12.15 \text{ mg/ml})(100 \text{ ml}) = x(5 \text{ mg/ml})$$

$$x = 243 \text{ ml}$$

- 100 ml

143 ml WFI pH 3.25

143 ml of WFI pH 3.25 were added to the GGF solution to achieve a 5 mg/ml solution. A 100ul aliquot was diluted 10X with WFI and its protein concentration was calculated using UV A_{280} .

To Page No. 81

Witnessed & Understood by me,

Pr Oil

Date

Invented by

Invented by Christine Jones

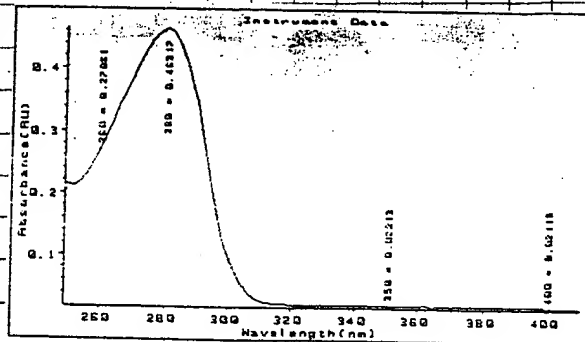
Recorded by

Date

TITLE

From Page No. 80

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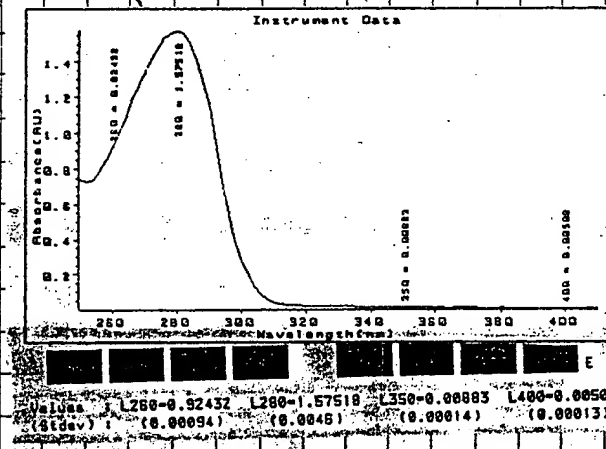
GRAPHICSD
(LABEL / HARDCOPY)

Values : L250=0.27061 L280=0.46317 L350=0.02213 L400=0.02113
 (Stdev) : (0.00023) (0.00035) (0.00012) (0.00013)

$$(.46317 A^{280} - .02213 A^{350}) / .86 = .513 \text{ mg/ml} \times 10 = 5.13 \text{ mg/ml}$$

This GCSF solution was sterile filtered with a .2 μ Costar filter Unit and stored at 4°C.

The GCSF solution of G from was placed into a 350ml Amicon stir cell w/ a YM10 100um membrane and concentrated to ~75ml. The solution was taken out and measured by weight into a beaker (150ml). It weighed 75.4g. A 100ul aliquot was diluted 10X w/ WFI and its protein concentration was calculated using UV A²⁸⁰ - A³⁵⁰.



To Page No. 82

Witnessed & Understood by me,

P. Ellis

Date

Invented by

Christine Farnon

Recorded by

Date

From Page No. 81

REDACTED

$$(1.57518 - 0.0883) / 0.86 \times 10 = 18.21 \text{ mg/ml}$$

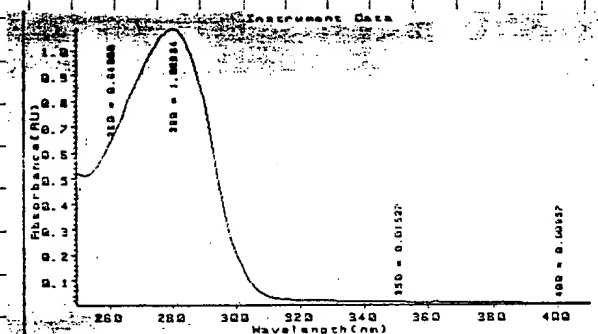
$$(18.21 \text{ mg/ml})(75.3 \text{ g}) = x(12.5 \text{ mg/ml})$$

$$x = 109.71 \text{ ml total}$$

- 75.30 ml GCSF

34.41 ml WFI pH 3.25

34.41 ml of WFI pH 3.25 was added to the GCSF solution to achieve a 12.5 mg/ml solution. Protein concentration was rechecked by A280:

GRAPHICSD
(LABEL / HARD COPY)

Values : L260=0.64000 L280=1.08884 L350=0.01527 L400=0.00957
(Stdev) : (0.00055) (0.0028) (0.00014) (0.00013)

$$(1.08884 - 0.01527) / 0.86 \times 10 = 12.48 \text{ mg/ml}$$

ADDING BICINE:

$$(109.71 \text{ ml})(12.5 \text{ mg/ml}) = (x \text{ ml})(10 \text{ mg/ml})$$

$$x = 137.14 \text{ ml total}$$

- 109.71 ml GCSF

27.43 ml of 500 mM Bicine pH 8

27.43 ml of 500 mM Bicine pH 8 was added to the 109.71 ml of 12.5 mg/ml GCSF solution in WFI pH 3.25 to achieve 137.14 ml of 10 mg/ml GCSF solution in 100 mM Bicine pH 8.0.

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Witnessed & Understood by me,

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Invented by

Date

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TITLE _____

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From Page No. 82

DETERMINING PEG AMOUNT:

$$(137.14 \text{ ml of GCSF}) (10^4 \text{ ng/ml}) = 1371.4 \text{ mg GCSF}$$

$$(1371.4 \text{ mg GCSF} / 8,800 \text{ ng/mol}) (1.5 \text{ molar ratio}) (6000 \text{ ng/mol PEG}) = 1156.52 \text{ mg PEG}$$

1156.52 mg of SCH-MPEG UCC lot 84-7 was weighed into a 700ml beaker. The GCSF solution was then added to the beaker and the reaction mixture was stirred for 1 hour. The solution was then diluted 5X w/ WFI pH 4.0-3.25.

$(137.14 \text{ ml of solution}) \times 5 = 685.70 \text{ total} - 137.14 \text{ ml solution} = 548.56 \text{ ml of WFI}$
After 548.56 ml of WFI pH 3.25 was added to the reaction solution the pH was adjusted to 3.25 and stored. The solution was sterile filtered using a Nalgene filter unit of 1 liter @ .45 μ and was stored at 4°C.

COLUMN PREPARATION:

A 45% slurry of ~700ml of Pharmacia S-Sepharose Fast Flow lot #036B was poured into a 1000ml beaker, allowed to settle, decanted, and mixed with milli-Q water to ~1000ml. This was repeated 5X. The resin was then poured into a Pharmacia XK50/30 column and packed at ~400 cm/hr with .5M NaCl in milli-Q water. When the bed height was low enough (after about 8 column volumes) the fitting was adjusted so that there was no head space. The column was then washed with 4 column volumes of deprogenate buffer (1M NaOH in WFI). The pH was then brought back down with 3 column volumes of 500mM NaOAc pH 4.0 and then re-equilibrated with 4 column volumes of 200mM NaOAc pH 4.0. The total column volume was calculated by the height of the bed and the circumference of the column:
 $\pi (2.5 \text{ cm})^2 (22.5 \text{ cm}) = 442 \text{ cm}^3 \text{ or } 442 \text{ ml}$

BUFFER PREPARATION:

116L of 20mM NaOAc pH 4.0 (A)

2L of 20mM NaOAc, 1M NaCl pH 4.0 (B)

To Page No. 84

Witnessed & Understood by me,

Date

Invented by

Christine Jamar

Date

Recorded by

P. C. C.

REDACTED

bead diameter

45 μ - 165 μ

average 90 μ

product # 17-0511-

S-Sepharose fast flow

id test
regulatory
01 TL
04 5L
60 60L

rom Page No. 82

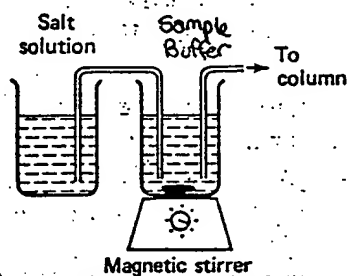
BLACK RUN (fractions marked w/ black pen):

METHOD:

equilibration ~ 3 col. vol. = 1300 ml (0% B)
 linear gradient ~ 18 col. vol. = 8000 ml (0% - 23% B)
 wash ~ 1 col. vol. = 450 ml (100% B)
 re-equilibration ~ 3 col. vol. = 1800 ml (0% B)

Flow rate: $(30 \text{ cm/hr})(2.5 \text{ cm})^2 \pi = 589 \text{ cm}^3/\text{hr} = \sim 10 \text{ ml/min}$

Because the available Pharmacia FPLC pumps were unable to sustain this flow rate (practice run done on 1/24/92 and discarded) a linear gradient was set up using the two beaker method w/ a magnetic flea to stir the sample buffer and a peristaltic pump to pump it through the column.



AT START OF RUN:

Salt Solution = $(8000 \text{ ml} / 2 \text{ beakers})(.23 \text{ of B}) =$
 900 ml B + 3080 ml A

Sample Buffer = $(8000 \text{ ml} / 2 \text{ beakers})(.00 \text{ B}) =$
 0 ml B + 4000 ml A

Volume (ml)

0.0
 0.0
 1300.0
 1300.0
 1300.0
 9200.0
 9650.0
 10000.0
 —
 10,000.0
 11,000.0

Instruction

EQUILIBRATION BUFFER (0% B)
 FLOW ON (10 ml/min)
 SAMPLE BUFFER (0% - 23% B)
 MONITOR ON (0.1 cm/ml)
 FRACTION COLLECTOR ON (1.5 min/fraction)
 WASH BUFFER (100% B)
 RE-EQUILIBRATION BUFFER (0% B)
 MONITOR OFF (0 cm/ml)
 FLOW OFF (0)
 FRACTION COLLECTOR OFF (0 min/fraction)
 FLOW OFF (0 ml/min)

To Page No. 85

Witnessed & Understood by me,

Grilles

Date

Invented by

Christine J. J. J.

Recorded by

Date

From Page No. 81

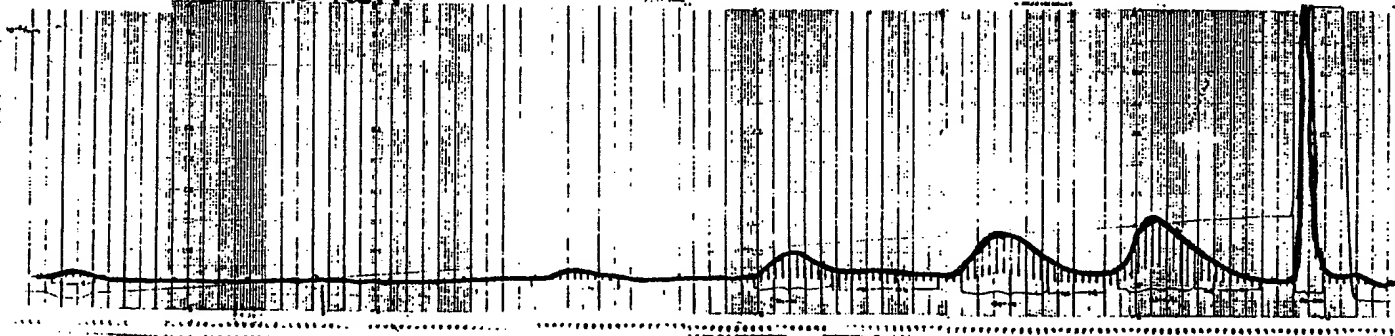
REDACTED

BLUE RUN (fractions marked w/ blue pen):

RUN:

220ml of 2 mg/ml 1.5X REG-GCSF from page 83 was loaded onto the 440ml S-Sepharose Column using the peristaltic pump. The method above was then used to elute the protein. The UV Monitor was set at 240nm. Fractions were collected in 50ml centrifuge tubes w/ an Advantec SF-2120 Super Fraction Collector, numbered, and stored at 4°C. (see fig. below)

BLACK RUN



BLUE RUN (fractions marked w/ blue pen):

METHOD:

The method was modified to shorten the run in light of the fact that "nothing" came off the column until the gradient reached ~10% B.

equilibration ~5 col. vol. = 1300 ml (0% B)

linear gradient ~15 col. vol. = 6600 ml (0% - 23% B)

wash ~1 col. vol. = 450 ml (100% B)

re-equilibration ~3 col. vol. = 1300 ml (0% B)

To Page No. 82

Witnessed & Understood by me,

Gr. Ellis

Date

Invented by

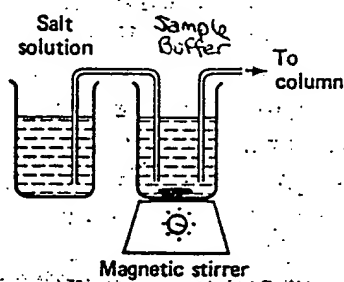
Christine Jordan

Date

Recorded by

From Page No. 85

The linear gradient was again set up using the two beaker method.



At start of RUN:

$$\text{Salt Solution} = (160 \text{ ml} / 2 \text{ beakers}) (.238) = 11.5 \text{ ml B} + 3.135 \text{ ml A}$$

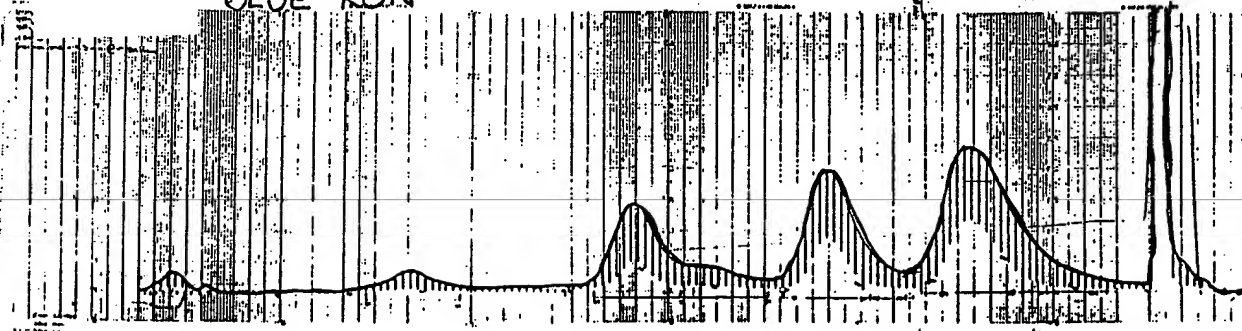
$$\text{Sample Buffer} = (160 \text{ ml} / 2 \text{ beakers}) (.58) = 75.9 \text{ ml B} + 2.541 \text{ ml A}$$

Volume (ml)	Instruction	
0.0	EQUILIBRATION BUFFER	(0% B)
0.0	FLOW ON	(10 ml/min)
1300.0	SAMPLE BUFFER	(5%-23% B)
1300.0	MONITOR ON	(.01 cm/ml)
1300.0	FRACTION COLLECTOR ON	(4.5 mm/fraction)
7900.0	WASH BUFFER	(100% B)
8350.0	RE-EQUILIBRATION BUFFER	(0% B)
8800.0	MONITOR OFF	(0 cm/ml)
9800.0	FLOW OFF	(0 ml/min)

RUN:

220 ml of 2 mg/ml 1.0X PEG 6000 from page 83 was loaded onto the 440 ml S-Sepharose column using the peristaltic pump. The method above was used to elute the protein. The UV Monitor was set at 1 AUFS. Fractions were collected in 50 ml centrifuge tubes w/ an Adventec SE-2120 Super Fraction Collector, numbered, & stored at 4°C. (See fig. below)

BLUE RUN



No. 81

Witnessed & Understood by me,

Rn Lee

Date

Invented by

Christine Jarrar

Date

Recorded by

REDACTED

From Page No. 86

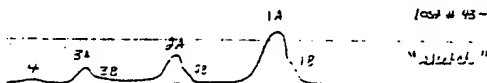
POOLING AND CONCENTRATING IEX FRACTIONS:

Fractions to be pooled were determined. A chart was drawn up by Elise Gabriel.

Prepared Peak Pools - mono - Poly GCSF Preparation

44um 1-sephacore HP column
run done on will not be include in pools.

peak id	black run 100148	blue run 120392
Peak 1A	152-162	109-123
1B	163-171	124-134
Peak 2A	130-141	90-101
2B	142-149	102-106
Peak 3A	101-111	64-75
3B	112-126	76-87
Peak 4	70-81	33-45 loss at 45-45



The black/blue runs will be to pool and concentrated in preparation for storage column. Will use Amicon stir cell and MWK mks.

Containers will be labeled with peak id and 10/17/99 date.

Pooling of IEX fractions done by Elise Gabriel.

started to concentrate peak 1A black pool before adding blue run with YM10 membranes and 40 psi (5.5 mL/min)

End of day - removed protein from stir cell and added WFF to membranes. Stored pools of protein + wet stir cell in refrigerator until tomorrow.

- took UV-vis scan of eluent + protein pool as day progressed. Scans are dated.

- discarded #1-20 of blue run

To Page No. 88

Witnessed & Understood by me,

Date

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Recorded by

Date

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From Page No. 87

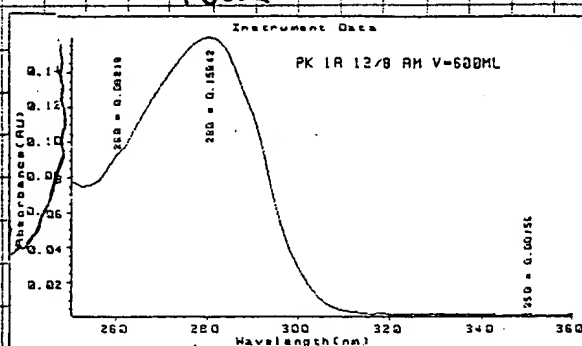
Concentration of Peaks 1A and 2A done by Elise Gabriel

Peak 2A was conc to less than 50 mL. The vol. was diluted to 100mL with 20mM NaOH pH 7.4 to Lemon Nuclei for storage

Peak 1A conc to 25mL. Added 20mM NaOH to 100mL and conc down again

1A:

POOL

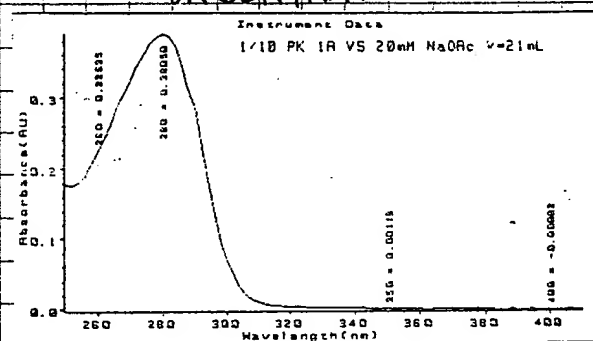
GRAPHICSD
(LABEL / HARD COPY)

$$(.15542^{280} - .00156^{360}) / 860 = .184 \text{ ng/mL}$$

$$\times 600 \text{ mL}$$

$$= 110 \text{ mg}$$

CONCENTRATE

GRAPHICSD
(LABEL / HARD COPY)

Values : L260=0.22635 L280=0.39050 L350=0.00119 L400=0.0002
(Stdev) : (0.00055) (0.00030) (0.00015) (0.00014)

$$(.39050^{280} - .0019^{360}) / 860 = 4.527 \text{ ng/mL}$$

$$\times 21 \text{ mL}$$

$$= 95 \text{ mg}$$

To Page No. 89

Witnessed & Understood by me,

Date

Invented by

Christine Jordan

Recorded by

Date

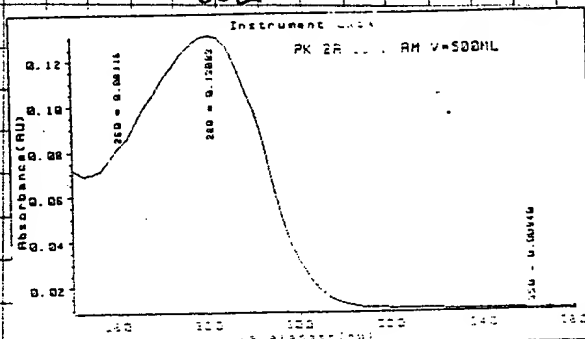
111

REDACTED

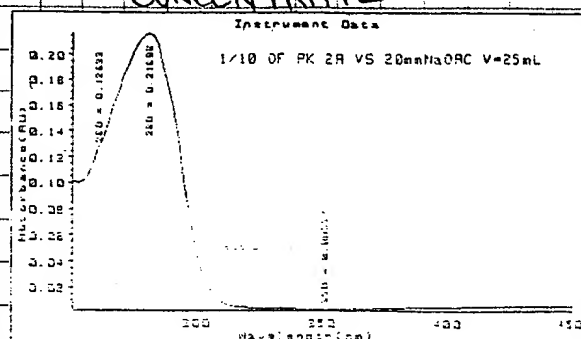
From Page No. 87

2A:

POOL



CONCENTRATE



GRAPHICSCO

(LABEL / HARD COPY)

33

GRAPHICSCO

(LABEL / HARD COPY)

33

Values : L260=0.12633 L280=0.21633 L350=0.00337
(Stdev) : (0.00032) (0.00019) (0.00015)

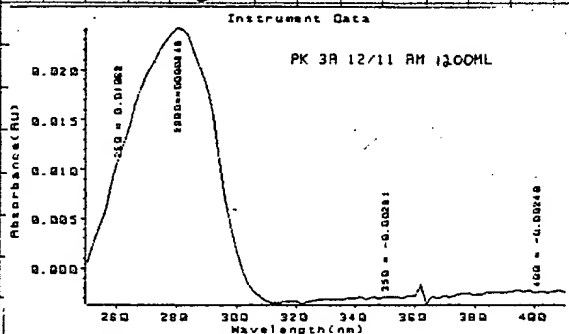
$$(0.12633^{A280} - 0.004^{A350}) / 86 = .14 \text{ mg/ml} \\ \times 500 \text{ ml} = 70.6 \text{ mg}$$

$$[(0.21633^{A280} - 0.00337^{A350}) / 86] 10 = 2.48 \text{ mg/ml} \\ \times 25 \text{ ml} = 62 \text{ mg}$$

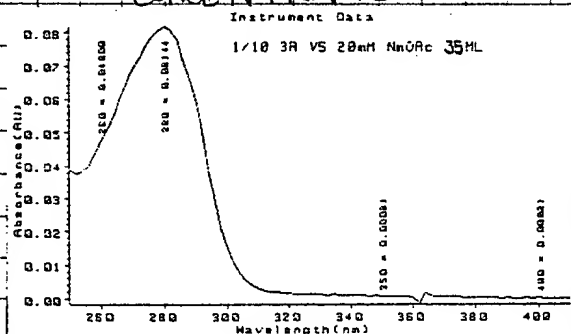
12-11-92

3A:

POOL



CONCENTRATE



GRAPHICSCO

(LABEL / HARD COPY)

33

GRAPHICSCO

(LABEL / HARD COPY)

33

Values : L260=0.01062 L280=0.02419 L350=0.00291 L400=0.0024
(Stdev) : (0.00032) (0.00016) (0.00010) (0.00010)

Values : L260=0.04000 L280=0.08144 L350=0.00081 L400=0.00021
(Stdev) : (0.00031) (0.00017) (0.00013) (0.00016)

$$(0.02419^{A280} - 0.00291^{A350}) / 86 = .0315 \text{ mg/ml} \\ \times 200 \text{ ml} = 6.3 \text{ mg}$$

$$[(0.08144^{A280} - 0.00081^{A350}) / 86] 10 = 9.376 \text{ mg/ml} \\ \times 35 \text{ ml} = 328 \text{ mg}$$

To Page No. 90

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Frutier

Christine Jones

From Page No. 89

Aliquots from the three concentrated peaks were run on an SEC3000 column:
on a Waters HPLC system in 100mM NaPhos pH 6.9:

Request # 1068

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: Chris FarmerProtein (Analyte): Peg-GCSFAnalysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CEColumn: SEC3000 #4930Method: 3004

Date Results Reported: _____

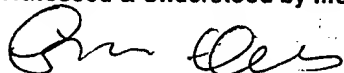
Instrument # 2

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	10	33-2474	1	STD	25				
2	15	2475	1	GCSF (stock)	26				
3	30	2476	1	"	27				
4	120	2477	1	"	28				
5	15	2478	2	15X AMN HIX	29				
6	7	2479	4.53	Peak #1A	30				
7	12	2480	2.48	Peak #2A	31				
8	32	2481	.74	Peak #3A	32				
9	30	2482	1	GCSF (stock)	33				
10	10	2483	1	STD	34				
11					35				
12					36				
13					37				
14					38				
15					39				
16					40				
17					41				
18					42				
19					43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 91

Witnessed & Understood by me,



Date

Invented by

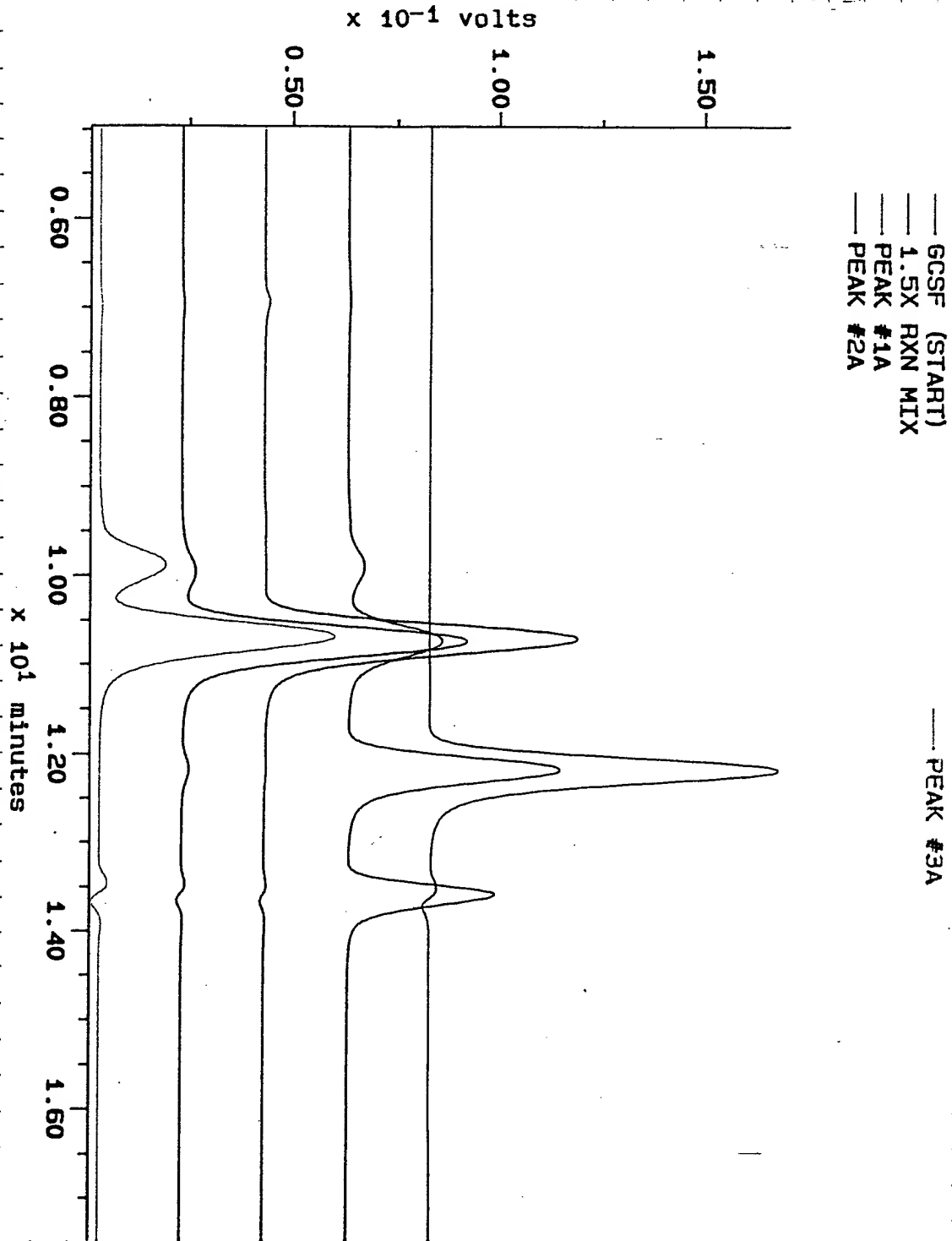
Christine Thomas

Date

Recorded by

TITLE _____

REDACTED

From Page No. 90To Page No. 92

Witnessed & Understood by me,

For Dec

Date

Invented by

Christine Farnan

Recorded by

Date

From Page No. 91

Aliquots from the three peaks were also run on 4-20% Gradient Mini Gel.

PEG-GCSF (1) 12/13/92

Date: _____

Operator: Chris

G-CSF gel I

NB No: 5575-40

4-20% Gradient Mini Gel

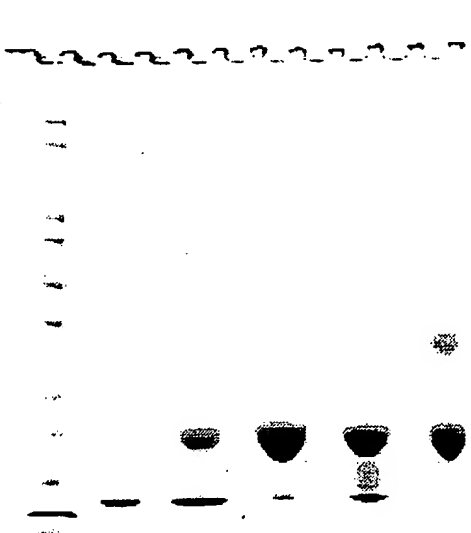
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF (START)	3	1.00	0.75	4.00	3.00
PEG-GCSF 1.5 RXN	5	2.00	1.50	6.67	10.00
PEAK #1A	7	4.53	3.40	2.95	10.00
PEAK #2A	9	2.48	1.86	5.38	10.00
PEAK #3A	11	0.94	0.70	14.22	10.00

To Page No. X

Witnessed & Understood by me,

Chris

Date

Invented by

Christine Farnham

Date

Recorded by

|||



Project No. _____

TITLE _____ Book No. 5574 93

From Page No. _____

REDACTED

To Page No. _____

Witnessed & Understood by me, 	Date :	Invented by	Date :
		Recorded by 	

From Page No. 2

REDACTED

To Page No. 4

Witnessed & Understood by me,

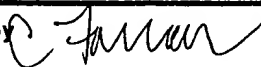


Date

Invented by

Date

Recorded by



|||

Project No. _____

TITLE _____

Book No. 5576

95

From Page No. X

REDACTED

To Page No. X

Witnessed & Understood by me,

Ph. Oes

Date

Invented by

Date

Recorded by


E. J. J. J.

From Page No. 1

REDACTED

To Page No. 4

Witnessed & Understood by me,



Date _____

Invented by 

Date _____

Recorded by _____

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LABORATORY
NOTEBOOK

No 6951

AMGEN.

REDACTED

6951

REC-51

Box 0614

REDACTED

NOTEBOOK NO. 6951
ISSUED TO Christine Foster
ON _____
DEPARTMENT 215
RETURNED _____ 19____

"MICROFILMED"
DATE _____

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

INSTRUCTIONS

1. The primary purpose of this notebook is to protect your and the Company's Patent-Rights by keeping records of all original work in a form acceptable as evidence if any legal conflict arises.
2.
 - When starting a page, enter the title, project number, and book number.
 - Use ink for permanence -- avoid pencil.
 - Record your work as you progress, including any spur-of-the-moment ideas which may be developed later.
 - Avoid making notes on loose paper to be recycled.
 - Record your work in such a manner that a co-worker can continue from where you stop. You might be ill and to protect your priority it could be urgent that the work continue while you are absent.
3.
 - Give a complete account of your experiments and the results, both positive and negative, including your observations.
 - Record all diagrams, layouts, plans, procedures, new ideas, or anything pertinent to your work including the details of any discussions with suppliers, or other people outside the Company.
 - Do not try to erase any incorrect entries; draw lines deleting them, note the corrections, sign and date the changes. This extra care is worthwhile because of the necessity of original data to prove priority of new discoveries.
4.
 - After entering your data, sign and date the entries.
 - Explain your work to at least two witnesses who are not co-inventors, and have them sign and date the pages in the place provided.
- Record the names of operators and witnesses present during any demonstration and have at least two witnesses sign the page. If no witnesses are present during an experiment of importance, repeat it in the presence of two witnesses.
5. Since computer programs can be patented these instructions apply to the development of computer software. In this case a description of the structure and operation of the program should be recorded in the notebook, together with a basic flow diagram which illustrates the essential features of the program. In the course of developing the code, the number of lines of code written each day should be recorded in the notebook, together with a statement of the portion of the flow diagram to which the section of code is directed.
6. This notebook and its contents are the exclusive property of the Company. It is confidential and the contents are not to be disclosed to anyone unless authorized by the Company. You must return it when completed, upon request, or upon termination of employment. It should be kept in a protected place. **If loss occurs, notify your supervisor immediately, and make a written report describing the circumstances of the loss.**

111

TITLE Large Scale Purification of 3 MONO PEG-GCSF Species Project No. 112053
Book No. 5951

1

From Page No. X

REDACTED

Materials: Pharmacia Superdex 75 HR 16/60 #9020015

MONO PEG-GCSF PEAK 1A Species (from NB 5576 pages 71-92)

MONO PEG-GCSF PEAK 2A Species (from NB 5576 pages 71-92)

MONO PEG-GCSF PEAK 3A Species (from NB 5576 pages 71-92)

1.5X PEG-GCSF Reaction Mixture (from NB 5576 page 83)

Pharmacia FPLC instrument

20mM NaOAc pH 4.0 buffer (made w/ WFI)

Centiprep 10 concentrating units from Amicon

Centricon 10 concentrating units from Amicon

4-20% Gradient Mini Gels from I.S.S.

Coomassie G250 solutions

Waters HPLC instrument

Phenomenex SEC 3000 BioSep column #4930

100mM NaPhos pH 6.9 (made w/ Milli Q)

To Page No. 1

Witnessed & Understood by me,

Arach. Gao

Date

Invented by

Arach. Gao
Recorded by

Date

From Page No. 1

REDACTED

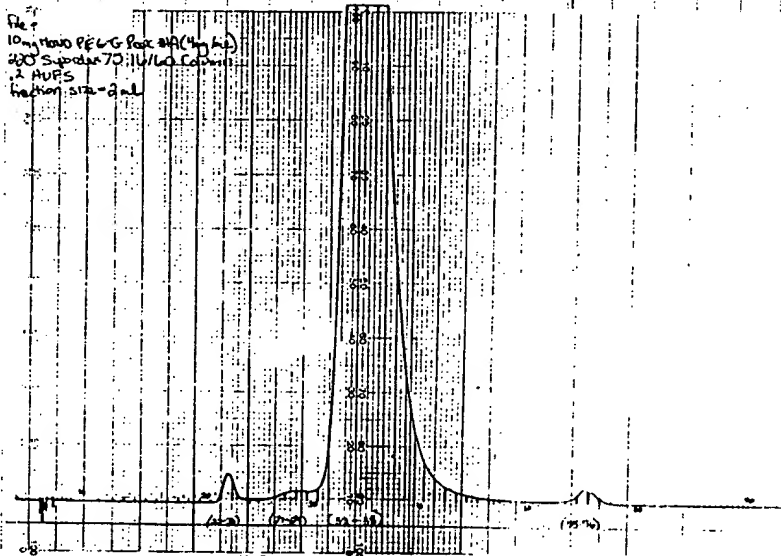
Procedure:

Method #1 from CEF (SPRDX75) was adjusted for a 120ml Superdex75 column:

Name = SPRDX75/120
 Bank = 1
 Number = 3

0.00	CONCAB	0
0.00	ML/MIN	1.5
0.00	VALVE.POS	1.1
0.00	VALVE.POS	3.1
0.00	MONITOR	1
0.00	LEVELA	1
0.00	CLEAR DATA	
0.00	VALVE.POS	1.2
0.00	CM/ML	.2
0.00	PORT.SET	6.1
5.00	VALVE.POS	1.1
150.0	PORT.SET	6.0
150.0	CM/ML	0
	END METHOD	

The 120ml Superdex75 was set up on the PLC and equilibrated in 20mM NaOAc pH 4.0. 10mg of Peak 1A was loaded onto a 2.5ml loop and eluted through the column using Method 5 from CEF. Fractions 22-23, 32-38, and 55-56 were collected, pooled separately, and stored at 4°C.

To Page No. 3

Witnessed & Understood by me,

Arnell H. Gaa

Date

Invented by

Arnell H. Gaa

Date

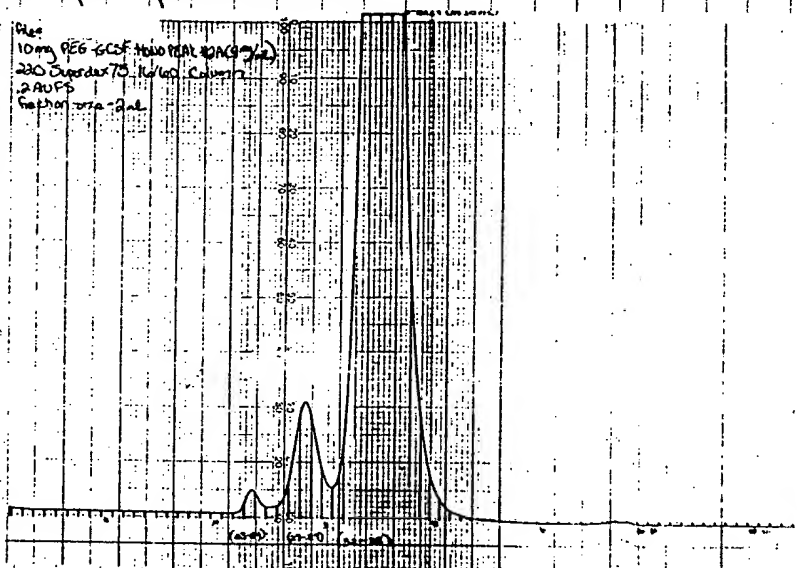
Recorded by

TITLE _____

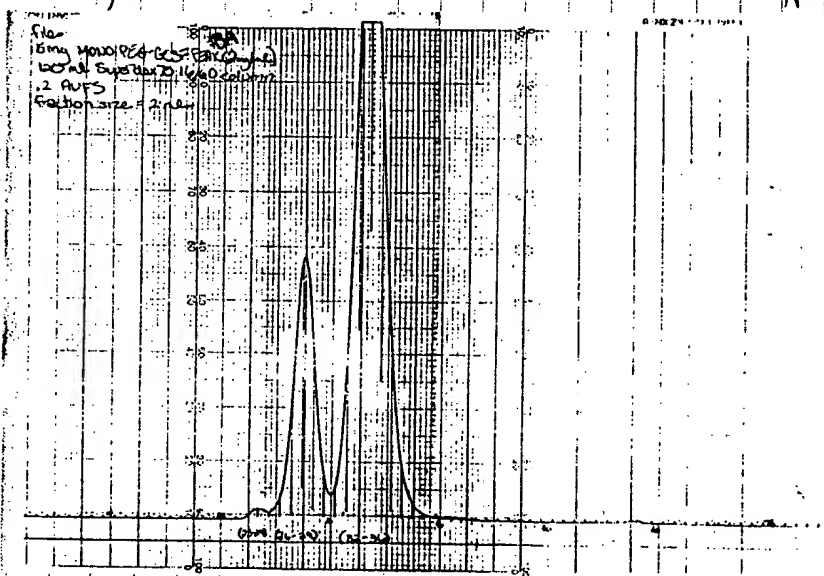
REDACTED

From Page No. 2

4ml of Peak 2A were concentrated in centrificon tubes to about 2.5ml at 4mg/ml. This was loaded onto a 2.5ml loop and eluted through the column using Method 3 from CEF. Fractions 23-24, 27-29, + 32-38 were collected and pooled separately, and stored at 4°C.



10ml of Peak 3A were concentrated in a centrificon 10 tube to about 5ml at 2mg/ml. 2.5ml of this was loaded onto a 2.5ml loop and eluted through the column using Method 3 from CEF. Fractions 23-24, 26-28, 32-34 were collected, pooled separately & stored @ 4°C.



To Page No. 4

Witnessed & Understood by me,

Date

Invented by

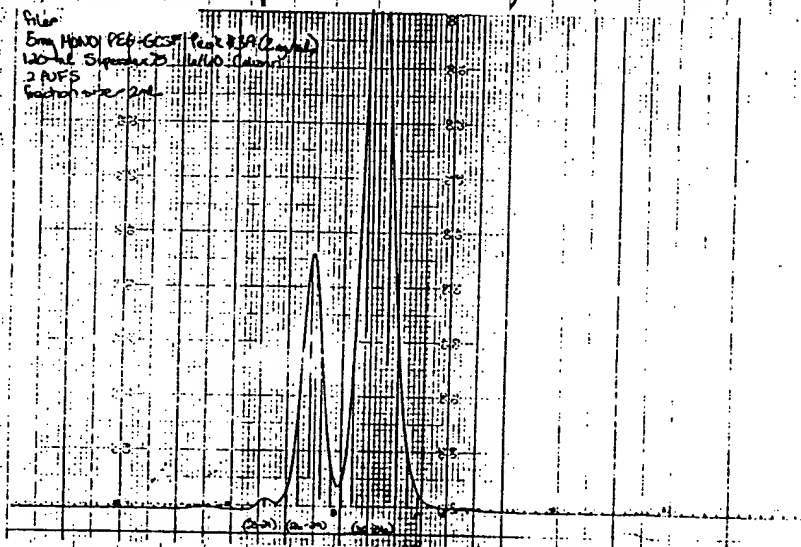
Christine Farnan

Date

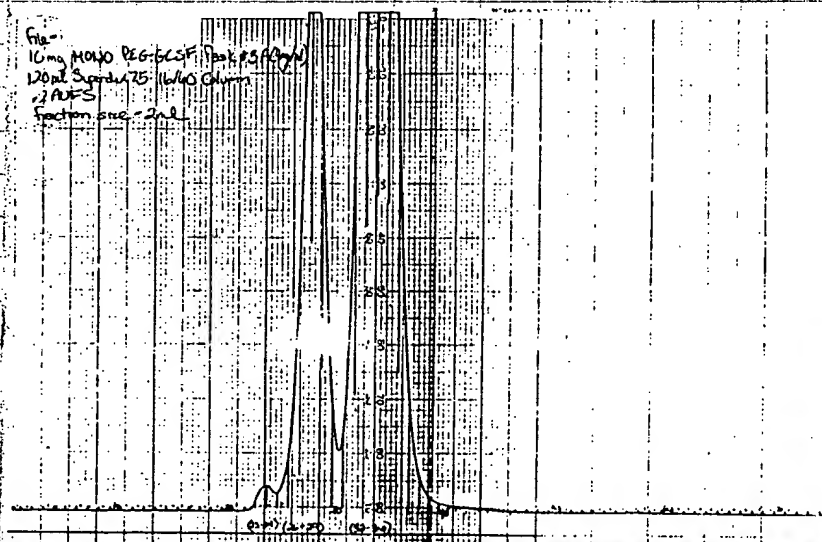
Recorded by

From Page No. 3

2.5ml of the concentrated Peak 3A from the previous page was loaded onto a 25ml loop and eluted through the column using Method 3 from CEF. Fractions 23-24, 26-29, & 32-36 were collected, & pooled w/ their peaks from, & stored at 4°C.



20ml of Peak 3A were concentrated in centriprep 10 tubes to about 5ml at ~4mg/ml. 2.5ml of this was loaded onto a 25ml loop & eluted through the column using Method 3 from CEF. Fractions 23-24, 26-29, & 32-36 were collected and pooled w/ the corresponding pools from, and stored @ 4°C.



To Page No. 8

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Arnell H. Gair

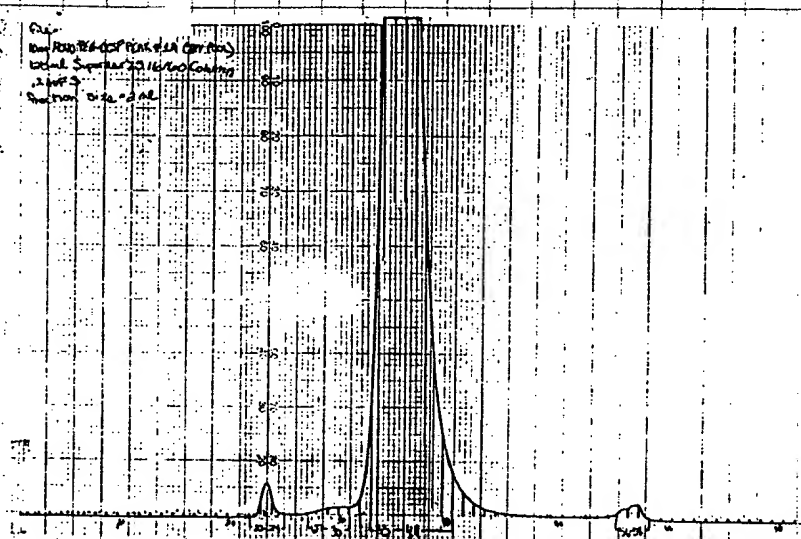
Christine Fagan

From Page No. 8

REDACTED

At this point, enough of Peak 1A, 2A, and 3A had been accumulated to run them on a gel, on an SEC HPLC column, and submit them for in vitro bioactivity assay and sequencing. The results from these tests are recorded on pages 14-18, 20-21.

10 µg of Peak 1A was loaded onto a 2.5 ml loop and eluted through the column using Method 3 from CEF. Fractions 22-24, 27-30, + 33-40 were collected & pooled w/ the corresponding peak 1A Superdex 75 pools, and stored at 4°C.



It was noted from the gel data and HPLC data on pages that there appeared to be unmodified GCSE in peak 2 even after SEC purification on the FPLC. Therefore, repurification of peak 2A on the FPLC was attempted. 4 mg of the Superdex 75 pool of peak 2A was loaded back onto the column. Fractions 32-39 were collected, pooled and stored @ 4°C. (figure on next page)

To Page No. 6

Witnessed & Understood by me,

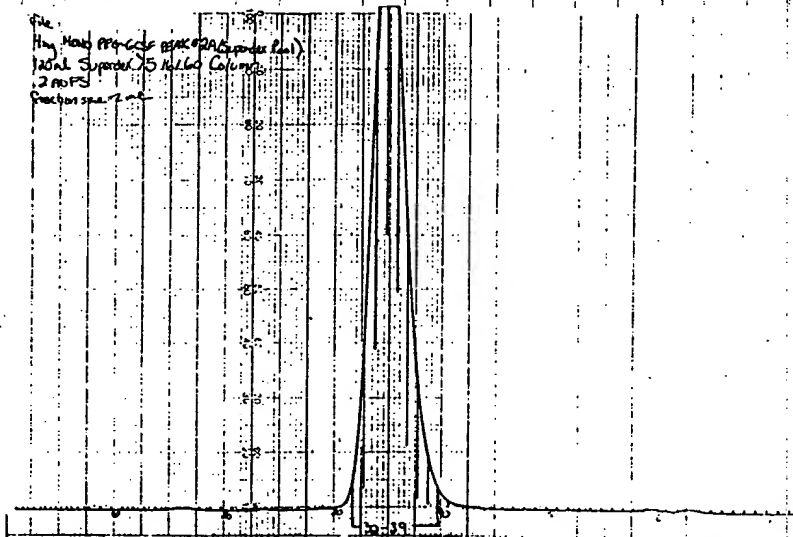
Date

Invented by

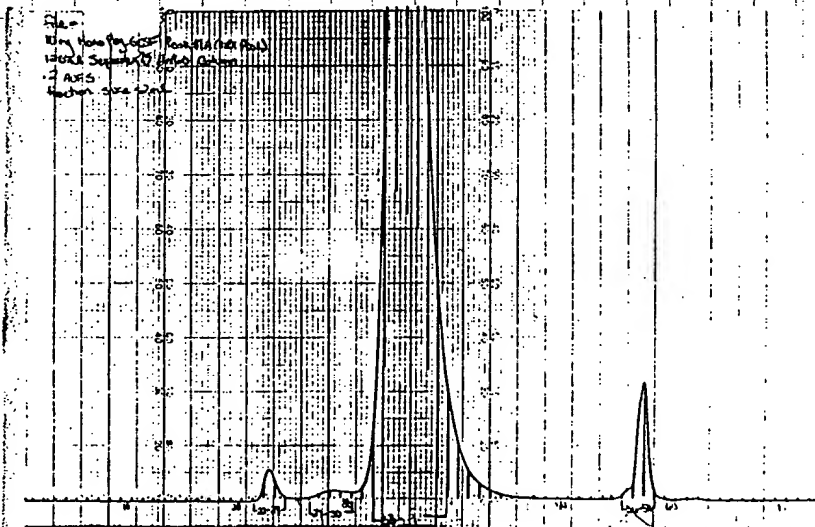
Date

Recorded by

*Arnell G. Gae**Christine Farrar*

From Page No. 5

10mg of Peak 1A was loaded onto a 2.5ml loop and eluted through the column using method 5 from C.F. Fraction 22-24, 27-30, 33-39, + 50-58 were collected & pooled with the previous corresponding Peak 1A Superdex 75 pools and stored at 4°C.

To Page No. 7

Witnessed & Understood by me,

Date

Invented by

Date

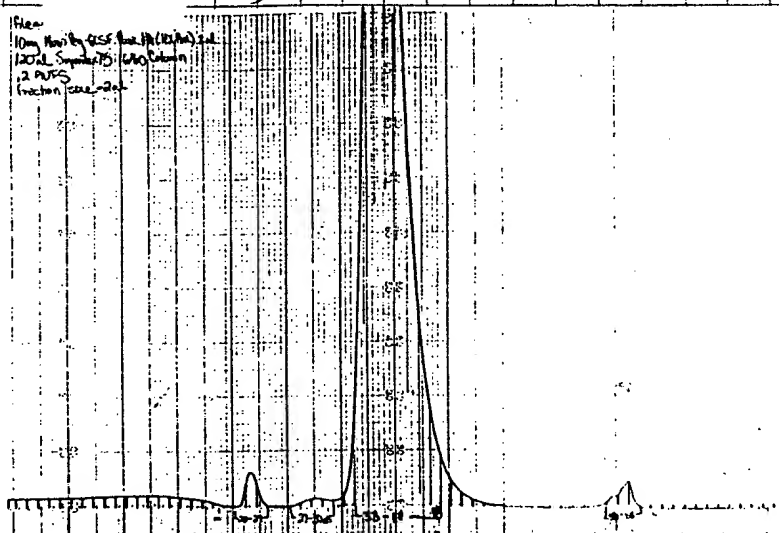
Recorded by

TITLE

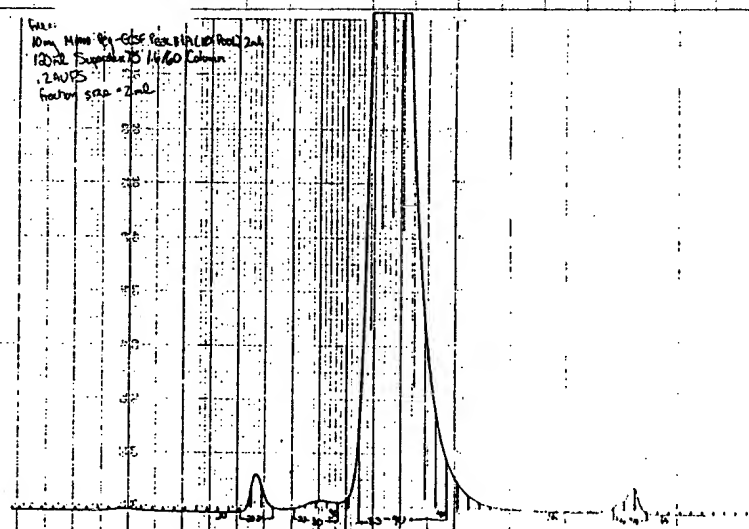
From Page No. 6

REDACTED

10mg of Peak 1A was loaded onto a 2.5ml loop and eluted through the column using method 3 from CEF. Fractions 22-24, 27-30, 33-40, 56-58 were collected, + pooled with the previous corresponding Peak 1A Superdex 15 pools + stored at 4°C.



10mg of Peak 1A was loaded onto a 2.5ml loop and eluted through the column using method 3 from CEF. Fractions 22-24, 27-30, 33-40, 56-58 were collected, + pooled with the previous corresponding Peak 1A Superdex 15 pools, + stored at 4°C.



To Page No. 9

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Tassar

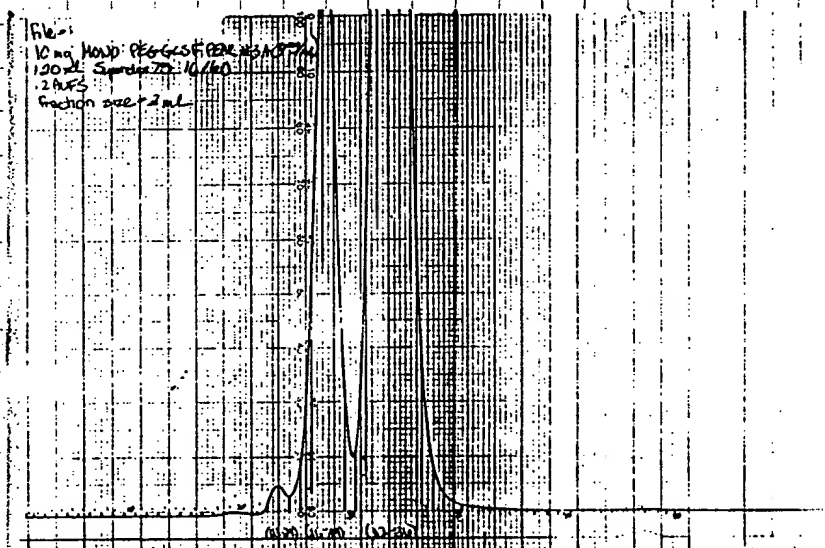
Date

Recorded by

From Page No. 4

REDACTED

2.5 ml of the concentrated Peak 3A from page 4 was loaded onto a 2.5 ml loop and eluted through the column using Method 3 from CEF. Fractions 23-24, 26-29, 32-36 were collected and pooled w/ the corresponding pools from. and stored @ 4°C.

To Page No. 5

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine J. Janssen

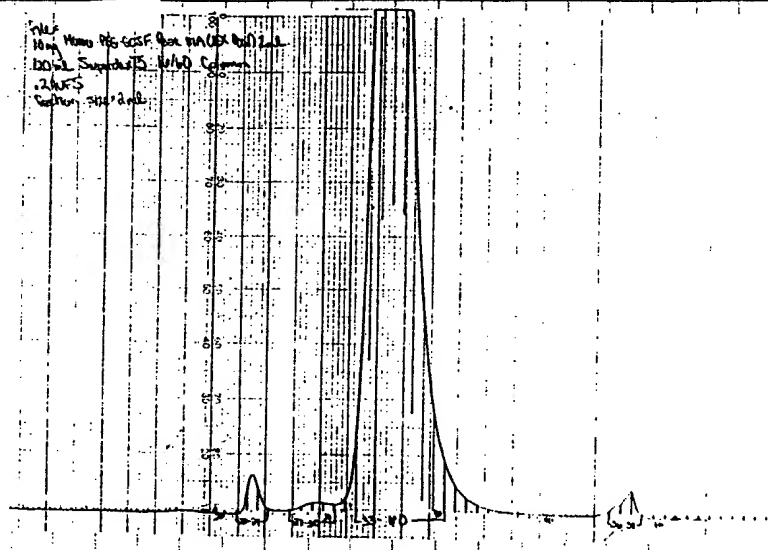
Date

Recorded by

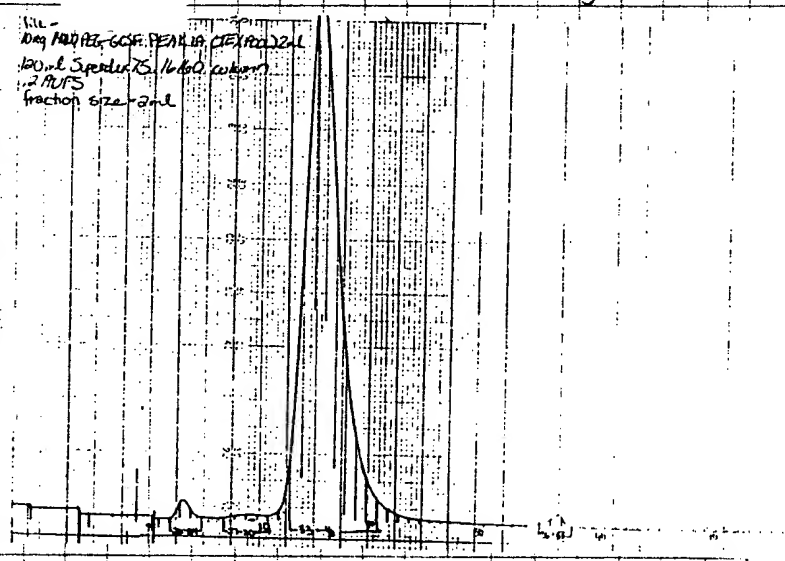
TITLE _____ REDACTED

From Page No. 1

10mg of Peak 1A was loaded onto a 25ml loop and eluted through the column using method 3 from CEF. Fractions 22-24, 27-30, 33-40, and 56-58 were collected & pooled with the previous corresponding Peak 1A Superdex 75 peaks & stored @ 4°C



10mg of Peak 1A was loaded onto a 2.5ml loop and eluted through the column using method 3 from CEF. Fractions 22-24, 27-30, 33-40 and 56-58 were collected & pooled with the previous corresponding Peak 1A Superdex 75 peaks & stored @ 4°C



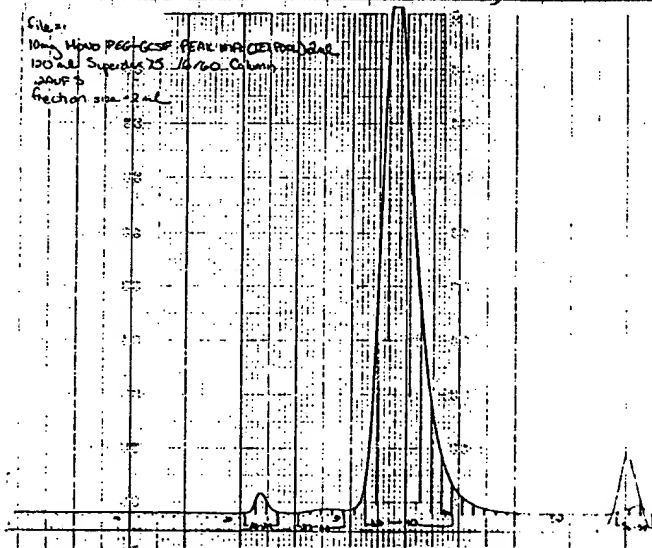
To Page No. 10

Witnessed & Understood by me, <i>Anne H. Lee</i>	Date	Invented by <i>Christine Farnon</i>	Date
		Recorded by	

From Page No. 9

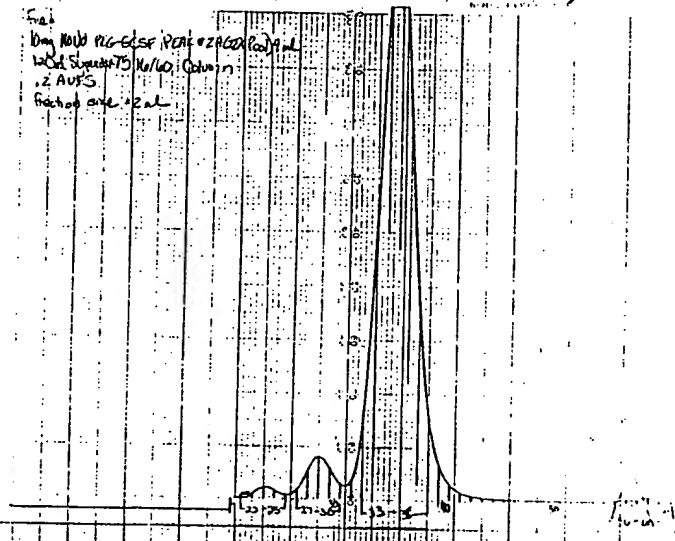
10 mg of Peak 1A was loaded onto a 2.5 ml loop and eluted through the column using method 3 from CEF. Fractions 22-25, 27-30, 33-38, and 51-62 were collected, pooled with the previous corresponding Peak 1A Superdex 75 pools, and stored @ 4°C.

File #:
10mg Mito PEG-CEP Peak 1A CEF Pool
100 ml Superdex 75 10/20 Column
2 AUFS
Fraction size: 2 ml



10 mg of Peak 2A was loaded onto a 4 ml loop and eluted through the column using method 3 from CEF. Fractions 22-25, 27-30, 33-38, and 51-62 were collected, pooled with the previous corresponding Peak 2A Superdex 75 pools, and stored @ 4°C.

File #:
10mg Mito PEG-CEP Peak 2A CEF Pool
100 ml Superdex 75 10/20 Column
2 AUFS
Fraction size: 2 ml

To Page No. 11

Witnessed & Understood by me,

Date

Invented by

Date

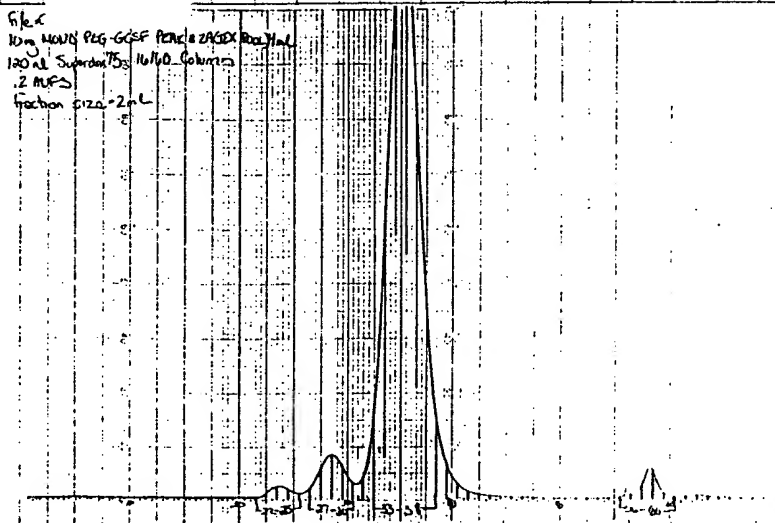
Recorded by

Arnell H. Gue

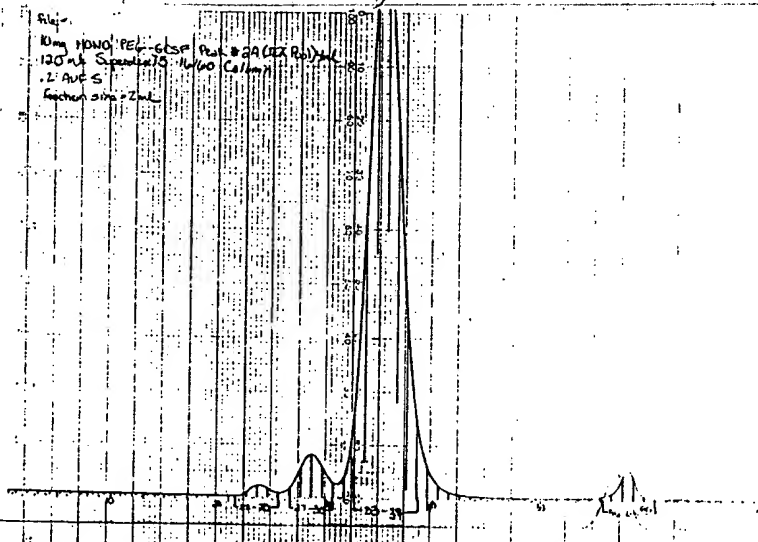
Christine J. Jones

From Page No. 10

10mg of Peak 2A was loaded onto a 4ml loop and eluted through the column using method 3 from CEF. Fractions 22-25, 27-30, and 33-38²⁰⁻⁶⁰ were collected and pooled with the previous corresponding Peak 2A Superdex 5 pools, & stored @ 4°C:



10mg of Peak 2A was loaded onto a 4ml loop and eluted through the column using Method 3 from CEF. Fractions 22-25, 27-30, and 33-34 and 51-60 were collected and pooled with the previous corresponding Peak 2A Superdex 5 pools, & stored @ 4°C:



To Page No. 12

Witnessed & Understood by me,

Anne H. Lee

Date

Invented by

Christine Farnas

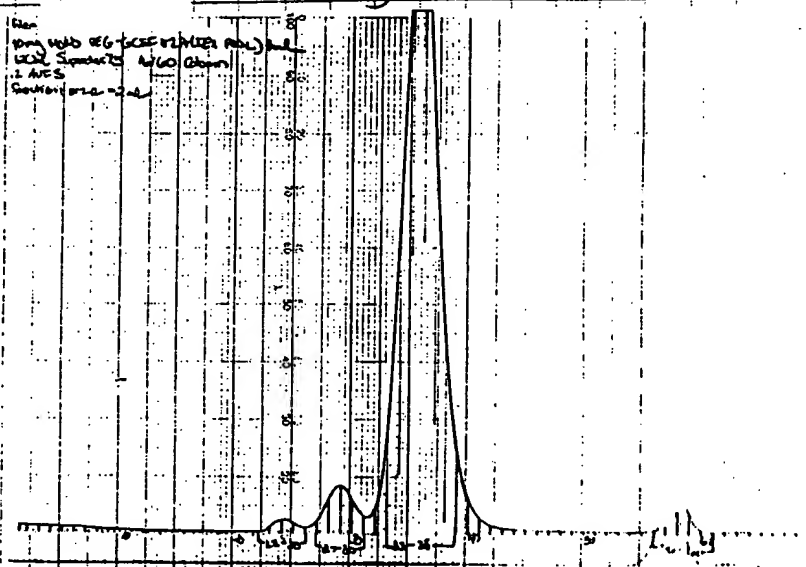
Date

Recorded by

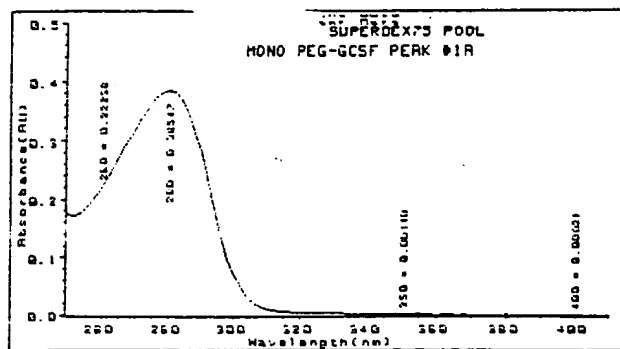
REDACTED

From Page No. 11

10 mg of Peak 2A was loaded onto a 4ml loop and eluted through the column using Method 3 from CEF. Fractions 27-30, 33-38, and 57-60 were collected and pooled with previous corresponding Peak 2A Superdex75 pools and stored @ 4°C.



An aliquot of 180 μ l was taken from each of the three peak solutions and total protein concentration of the three mono pegylated species was calculated using $A_{280} \times f = .86$:



.4466 mg/ml

GRAPHICS
[LABEL / HARD COPY]

Values : L260=0.22238 L280=0.38547 L350=0.00140 L400=0.00021
(Stdev) : (0.00027) (0.00056) (0.00019) (0.00022)

PEAK #1A

$$(.38547^{A_{280}} - .0014^{A_{350}}) / .86 = .4466 \text{ mg/ml}$$

$$(.4466 \text{ mg/ml}) (4 \text{ ml}) = 1.786 \text{ mg}$$

To Page No. 13

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

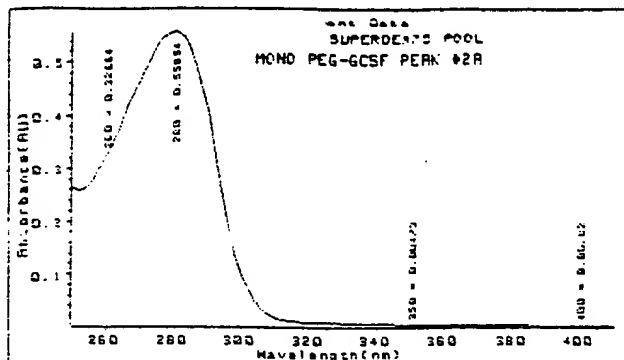
Anne H. Gar

Christine Furrer

TITLE

REDACTED

From Page No. 12

GRAPHICSD
(LABEL / HARD COPY)

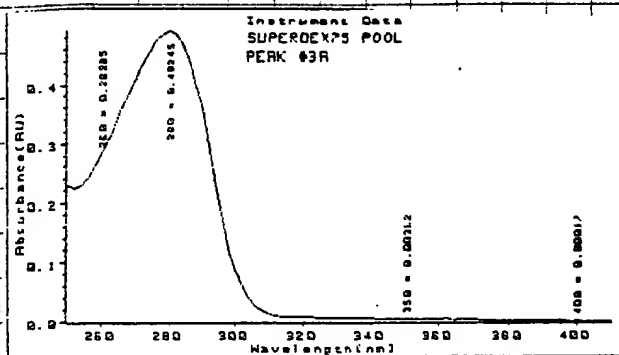
35
E

Values : L260=0.32664 L280=0.55954 L350=0.00473 L400=0.00282
(Stdev) : (0.00023) (0.00055) (0.00020) (0.00017)

PEAK #2A

$$(0.55954 \text{ AU} - 0.00473 \text{ AU}) / 86 = 0.0064 \text{ mg/ml}$$

$$(0.0064 \text{ mg/ml}) (48 \text{ ml}) = \sim 31 \text{ mg}$$

GRAPHICSD
(LABEL / HARD COPY)

35
E

Values : L260=0.32885 L280=0.49245 L350=0.00362 L400=0.00017
(Stdev) : (0.00029) (0.00051) (0.00016) (0.00012)

PEAK #3A

$$(0.49245 \text{ AU} - 0.00362 \text{ AU}) / 86 = 0.0056 \text{ mg/ml}$$

$$(0.0056 \text{ mg/ml}) (34 \text{ ml}) = \sim 19 \text{ mg}$$

To Page No. 14

Witnessed & Understood by me,

Anne H. Lee

Date

Invented by

Christine Turner

Recorded by

Date

REDACTED

From Page No. 13SDS PAGE GEL

Aliquots from the three peaks were run on SDS/PAGE Gradient Mini Gel using the SOP for Coomassie stained mini gels.

PEG-GCSF

Date: _____

Operator: Chris

G-CSF gel 1

NB No: 5575-42

4-20% Gradient Mini Gel

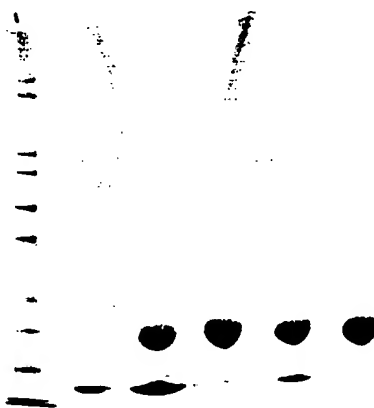
Running Conditions

constant current: 25 mA

all non-reduced

Coomassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF (START)	3	1.00	0.75	4.00	3.00
PEG-GCSF 1.5 RXN	5	2.00	1.50	6.67	10.00
PEAK #1A	7	0.57	0.42	23.57	10.00
PEAK #2A	9	0.55	0.42	24.03	10.00
PEAK #3A	11	0.57	0.43	23.46	10.00



Page 1

To Page No. 15

Witnessed & Understood by me,

Anne H. Gao

Date

Invented by

Christine Farnon

Date

Recorded by

From Page No. 14

HPLC OVERLAYS

Aliquots from the three peaks were run on the Waters HPLC using a Phenomenex SEC 8000 column # 4930.

Request # 1057

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: CF

Protein (Analyte): PEG-GCSF

Analysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CF

Column: SEC3000 # 4930

Method: 3009

Date Results Reported: _____

Instrument # 2

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	10	85-253	1	STD	25				
2	15	254	1	GCSF	26				
3	30	255	1	"	27				
4	60	256	1	"	28				
5	20	257	1	PEG (0.113-24)	29				
6	40	258	1	"	30				
7	60	259	1	"	31				
8	20	260	1	PEG (84-7)	32				
9	40	261	1	"	33				
10	60	262	1	"	34				
11	10	263	3	PEG-GCSF 8X	35				
12	11	264	2.7	" 16X	36				
13	10	265	3	4H PEG 16X	37				
14	1.5	266	2	1.5X RXN MIX	38				
15	17	267	.564	MIX PEG-GCSF #1	39				
16	17	268	.9548	MIX PEG-GCSF #2	40				
17	17	269	.5684	MIX PEG-GCSF #3	41				
18	30	270	1	GCSF	42				
19	10	271	1	STD	43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 16

Witnessed & Understood by me,

Date

Invented by

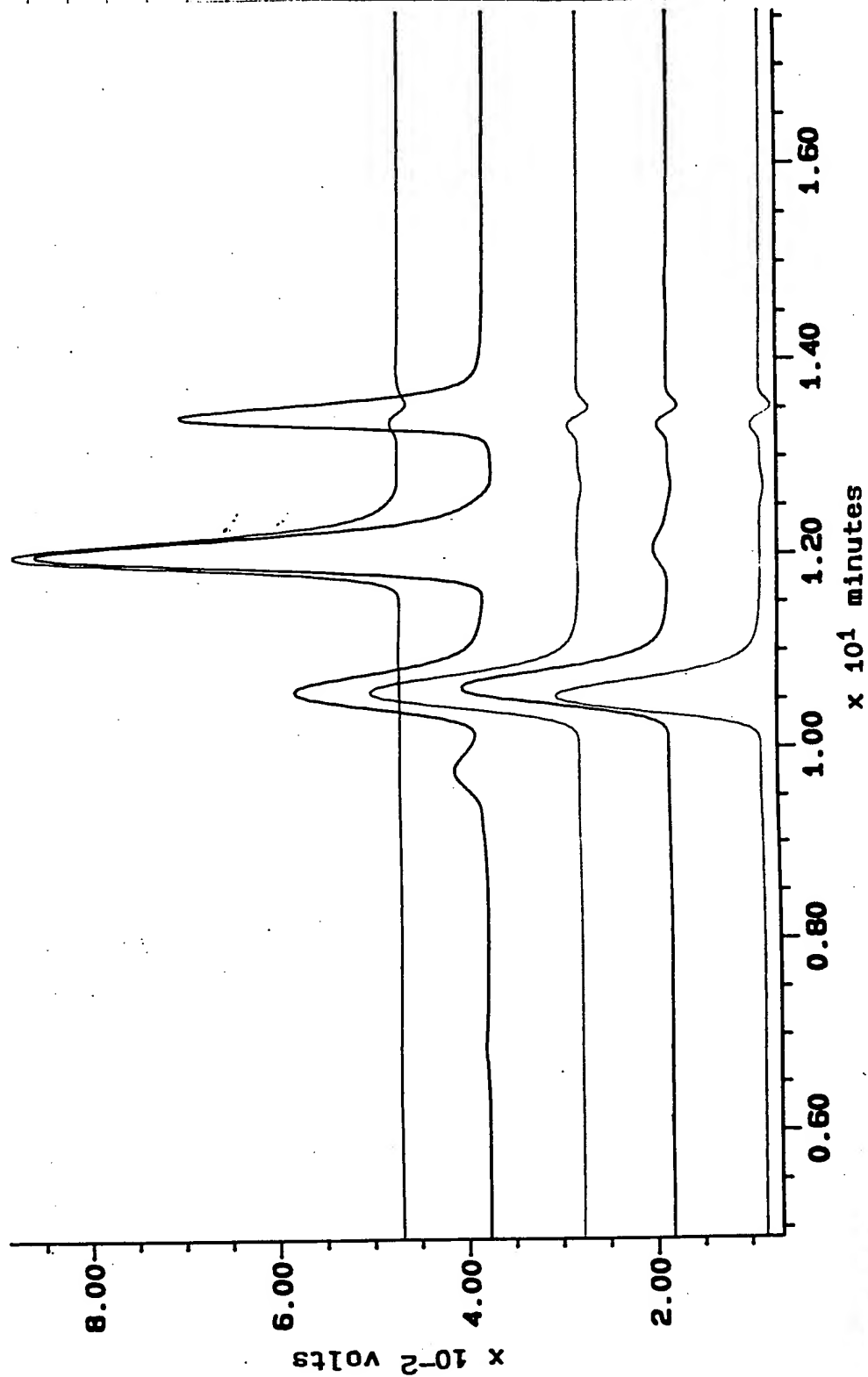
Date

Recorded by

REDACTED

From Page No. 15

— PEAK #3A SEC

— 6CSF
— 1.5X RXN MIX
— PEAK #1A SEC
— PEAK #2A SECTo Page No. 17

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

TITLE

From Page No. 10

INVITRO BIOASSAY

REDACTED

1 ml Aliquots of the 3 MONO-PEG-GCSF Species ~ 5 mg/ml was submitted to Wendy Griener in Analytic Services Department for invitro bioassay:

ASSAY DATE				Standard curve format			
SAMPLE				mg/ml from A-280	INITIAL DILUTION	U/ml assayed x E+08	U/mg calculated x E+08
1	630EOC CTRL	SO142		0.29	5 X E4	0.31	1.07
2	6703M2A	SO348	4°C #2 3CC GVT	0.29	5 X E4	0.32	1.09
3	6703M2A	SO348	4°C #1 3CC GVT	0.29	5 X E4	0.35	1.20
4	6703M2	SO348	4°C #2 3CC GVT	0.29	5 X E4	0.34	1.18
5	6703M2	SO348	4°C #1 3CC GVT	0.29	5 X E4	0.33	1.13
6	6703L2	SO347	4°C #2 3CC GVT	0.30	5 X E4	0.35	1.18
7	6703L2	SO347	4°C #1 3CC GVT	0.30	5 X E4	0.38	1.25
8	692A	SO140	9 MON. 4°C 15CC PPS	2.94	5 X E5	3.11	1.06
9	692B	SO140	9 MON. 15CC PPS	2.95	5 X E5	2.89	0.98
10	619G82	SO142	48 MON. 4°C 2CC GVT	0.31	5 X E4	0.31	1.01
11	619G82	SO142	48 MON. CRT 2CC GVT	0.31	5 X E4	0.23	0.78
12	683	SO140	12 MON. 4°C 1.2 CORN	2.80	5 X E5	3.14	1.12
13	683	SO140	12 MON. -80°C 1.2 CORN	2.80	5 X E5	3.09	1.10
14	630EOC CTRL	SO142		0.29	5 X E4	0.38	1.29
15	683	SO140	12 MON. LN2 VP 1.2 CORN	2.80	5 X E5	4.00	1.43
16	680	SO140	18 MON. 4°C U 20CC GVT	2.90	5 X E5	2.97	1.02
17	652L0	SO229	24 MON. 4°C TW SYR T	0.29	5 X E4	0.42	1.44
18	652L0	SO229	24 MON. CRT TW SYR T	0.29	5 X E4	0.26	0.90
19	GCSF (START)		CHRIS FARRAR	0.50	5 X E4	0.68	1.35
20	GCSF (CONTROL)		CHRIS FARRAR	0.04	5 X E3	0.03	0.73
21	R2N MX		CHRIS FARRAR	0.50	5 X E4	0.40	0.80
22	MONO PEG-G (1)		CHRIS FARRAR	0.50	5 X E4	0.46	0.92
23	MONO PEG-G (2)		CHRIS FARRAR	0.50	5 X E4	0.38	0.75
24	MONO PEG-G (3)		CHRIS FARRAR	0.50	5 X E4	0.15	0.29
25	MONO PEG-G (4)		CHRIS FARRAR	0.50	5 X E4	0.46	0.91
26	MONO PEG-G (5)		CHRIS FARRAR	0.50	5 X E4	0.38	0.78
27	MONO PEG-G (6)		CHRIS FARRAR	0.50	5 X E4	0.13	0.28
28	630EOC CTRL	SO142		0.29	5 X E4	0.24	0.82
630EOC AVERAGE OF 3 =				1.06 X E-08 U/MG			
U = NBSC STANDARDIZED UNITS							

INVITRO BIOASSAY RESULTS PERFORMED BY WENDY GRIENER AND LISA ARBOGAST

BIOACTIVITY OF MONO PEG-GCSF SPECIES



To Page No. 18

Witnessed & Understood by me,

Anne H. Lee

Date

Invented by

Christine Farrar

Date

Recorded by

From Page No. 17

IN VIVO BIOASSAY

The Mono-Peg-GCSF Species were bufferexchanged into GCSF formulation buffer (p. 23-26) and an aliquot of each was diluted to 1 mg/ml along w/ an aliquot of GCSF lot T6702. The 1 mg/ml Species, GCSF, and some of the GCSF formulation buffer were sterile filtered and transferred into sterile injection vials (5ml) & sealed with 13mm septa seals all performed in a laminar flow hood. The samples were then submitted to Lane Whitcomb of the Pharmaceuticals Department for in vivo bioassay.

To: Lane Whitcomb
 From: Elise Gabriel and Chris Farrar
 Subject: Mono-Peg-GCSF Hamster Dosing Study No. G021593
 Date: Monday,

Mono-Peg-GCSF Hamster Dosing Study No. G021593Animals:

Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule:

single S.C. injection of 0.1 mL on the first day

Sacrifice Schedule:

Four animals from each group will be bled at
 0.5, 1.0, 1.5, 2.0, 4.0, and 7.0 days following dosing.
 (Please note time if different than listed.)

Injection Notes:

Use one vial for each treatment group.
 Fill a 1-mL syringe, and inject up to 10 hamsters.
 No need to change syringe needles between hamsters
 within a single group.
 Vials are overfilled by 1.1 mL to allow for losses during
 filling syringes.

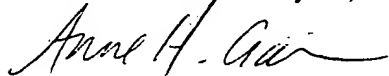
Analysis:

- Complete Blood Count: to be done on the same day that the
 samples were collected. Samples exceeding the range of the blood cell
 counter (other than platelets) will be diluted and recounted.
 - Blood smear slides: a thin, air-dried blood smear slide will be
 prepared and stained with a stain (Wright or equivalent) suitable for
 differential leukocyte analysis.

Group	Animal per Group	Injection mL	Aver. Wt. (Kg)	Vial Vol. (mL)	No. of Vials
1. Vehicle	24	0.1	0.1	3.5	1
2. 6951-23 peak 1	24	0.1	0.1	3.5	1
3. 6951-24 peak 2	24	0.1	0.1	3.5	1
4. 6951-25 peak 3	24	0.1	0.1	3.5	1
5. GCSF T6702	24	0.1	0.1	3.5	1

To Page No. 19

Witnessed & Understood by me,



Date

Invented by



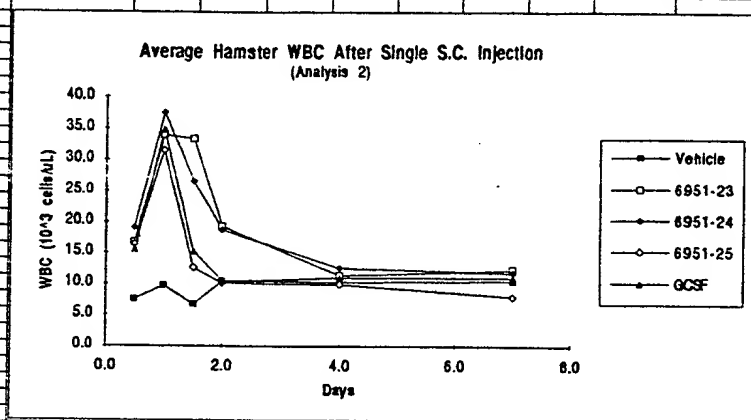
Date

Recorded by

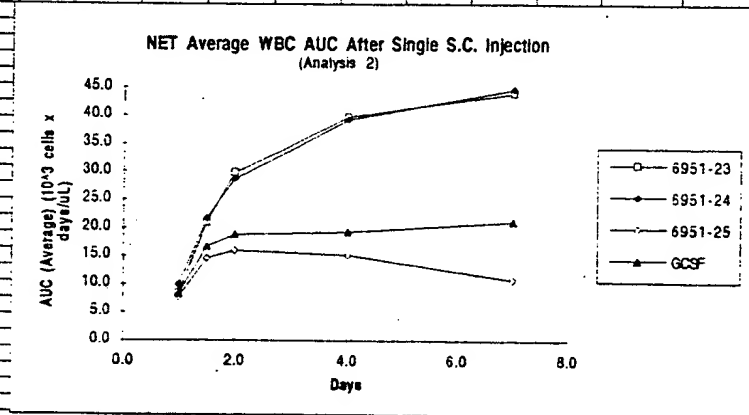
From Page No. 18

Analysis of data and graphs by Elise Gabriel: showing that regulated N-terminus and Lysine 35 species have the highest in vivo activity and that regulated Lysine 41 has decreased in vivo activity.

Table 1: Average WBC per Time Point (Days)							
Group No.	Sample Code/Days	0.5	1.0	1.5	2.0	4.0	7.0
1	Vehicle	7.5	9.7	6.8	10.5	10.3	10.4 #DIV/0!
2	6951-23	14.6	33.9	33.2	19.3	11.3	12.2 #DIV/0!
3	6951-24	14.0	37.5	26.5	18.7	12.8	11.7 #DIV/0!
4	6951-25	18.4	31.4	12.5	10.0	9.9	7.9 #DIV/0!
5	GCSF	15.5	34.7	15.2	10.3	11.0	11.0 #DIV/0!



Group No.	Sample Code/Days	1.0	1.5	2.0	4.0	7.0
2	6951-23	8.3	21.0	29.9	39.8	44.0 #DIV/0!
3	6951-24	9.8	21.7	28.8	39.3	44.7 #DIV/0!
4	6951-25	7.7	14.6	16.0	15.2	10.9 #DIV/0!
5	GCSF	8.3	18.7	18.8	19.4	21.2 #DIV/0!



To Page No. 20

Witnessed & Understood by me,

Arnold G. G.

Date

Invented by

Christine Jordan

Date

Recorded by

From Page No. 1 SEQUENCE ANALYSIS

1 ml Aliquots of the 3 MONO-PEG GCSF Species (p5) ~ 5mg/ml
was submitted to Chris Clogston in Protein Chemistry Department
for sequence analysis.

AMGEN

MEMORANDUM

To: Chris Farrar and Elise Gabriel *cc*From: Lee Anne Mercweather and Chris Clogston, and Hsieng Lu *HL*

Date: _____

Subj: GCSF Mono-PEG Forms

N-terminal sequence analysis

N-terminal sequence analysis was performed on the three samples submitted to this lab: GCSF mono-PEG 1, 2, and 3. Approximately 500 pmol was loaded onto an Applied Biosystems 477A protein sequencer and run for 25 cycles according to Analytical Method A0102. The sequence detected for all three samples was that of r-methuGCSF. Mono-PEG 1 shows a 5% initial yield indicating that its N-terminal group has been highly pegylated. GCSF Mono-PEG 2 and 3 show much higher yields at the N-terminus and lysine residues 17 and 24 thus indicating no detectable pegylation at these sites.

Reductive alkylation, Endoproteinase SV8 digestion and HPLC peptide mapping

500 ug aliquots of mono-PEG GCSF were Speed Vac dried and then reconstituted (1mg in 950ul) in 0.3M Tris-HCl containing 6M GdnHCl and 1mM EDTA, pH 8.4. Samples were then reduced by adding dithiothreitol and incubated at 37°C for 15 minutes. Samples were then S-carboxymethylated by adding iodoacetic acid and incubated at 37°C for 20 minutes. Samples were then desalted using Sephadex G-25 Quick Spin Protein Columns and buffer exchanged. After desalting and buffer exchange, sample concentration was adjusted to 0.5 mg/ml using additional buffer. Samples were then digested with SV8 (enzyme to substrate ratio 1:25) at 25°C for 26 hours. Protein digests were injected onto a Vydac C4 column (4.6 x 250mm, 5µ particle size, 300Å pore size) and peptides mapped by HPLC using a linear gradient of acetonitrile in 0.1% TFA. Peptides were manually collected and dried in a Speed Vac for sequence analysis.

N-terminal sequence analysis of SV8 peptides

In the GCSF 683 (reference std) SV8 map, the N-terminal peptide eluted at 57.3 minutes while in the mono-PEG 1 SV8 map, this peptide peak height dramatically diminished and a new peptide appeared as a broader peak at 77.5 minutes. The GCSF mono-PEG 2 SV8 peptide map showed a decrease in peak height (~40%), for a peptide with a retention time of 30.3 minutes, and a new peptide eluted at 66.3 minutes. The GCSF mono-PEG 3 peptide map was missing the peptide at retention time 30.3 minutes with a new peptide eluting at 66.4 minutes. These differences reflect the change in hydrophobicity due to pegylation. Each of the "new" peptides in the above maps were N-terminally sequenced for identification. These peptides were the only significant differences in the sample maps. There are some small incomplete cleavages seen on either side of the peptide at 86.1 minutes due to minor digestion differences (See Figures 1-3).

The dried peptides were reconstituted in 0.1% TFA and sequenced on an ABI protein sequencer. 60% of the total peak volume collected for mono-PEG 1 peptide 77.5 minutes was sequenced for 10 cycles. The sequence detected was that of the N-terminal peptide, M-T-P-L-G-P-A-S-S- with an estimated initial yield of less than 5%, suggesting that the N-terminal Met is blocked by a PEG molecule. It is noted that this PEG-peptide should result in a zero initial yield. The <5% yield we observed may be from detachment of PEG from the N-terminal Met during sequence analysis.

80% of the total peak volume collected for mono-PEG 2 peptide 66.3 minutes was sequenced 9 cycles. The sequence detected was K-L-C-A-T-Y-K-L-. This peptide contains lysine residues 35 and 41. The recovery of lysine 35 was significantly low, indicating pegylation at position 35. The recovery of lysine 41 was consistent with the other residues, indicating no modification of this position. The peptide at 30.3 minutes decreased in peak height compared to the corresponding peak in the reference standard map. This peptide is only 57.5% the peak area of the corresponding peptide.

80% of the total peak volume collected for mono-PEG 3 SV8 peptide 66.4 minutes was sequenced 9 cycles according to Analytical Method A0102. The sequence detected was K-L-C-A-T-Y-K-L-, and contained lysine residues 35 and 41. The recovery of lysine 35 was consistent with the other residue recoveries. The recovery of lysine 41 was significantly lower indicating pegylation at position 41 (See Figures 4-6).

Results: Mono-PEG 1: N-terminus pegylated

Mono-PEG 2: Lysine 35 partially pegylated

Mono-PEG 3: Lysine 41 pegylated

By comparing both reference standard and GCSF mono-PEG 1, 2, and 3 peptide maps, we found that both the mono-PEG 2 and mono-PEG 3 maps exhibit slightly diminished peak heights for the N-terminal peptide. This suggests that GCSF mono-PEG 2 and 3 contain a small amount of mono-PEG 1 contamination, or that their N-terminal methionine has a small percentage of pegylation.

(from conversation w/ Lee Anne Mercweather (3255))
Notes: Mono Peg #1 - 5% yield; saw methionine signal ~1% pmol (could actually be more), spotted 500 pmol
Not Quantitative (never 100%), 75% initial yield.

To Page No. 21

Witnessed & Understood by me,

Date _____

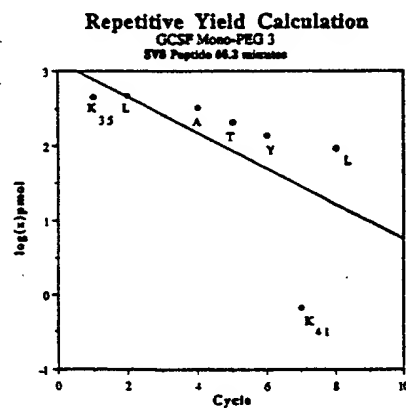
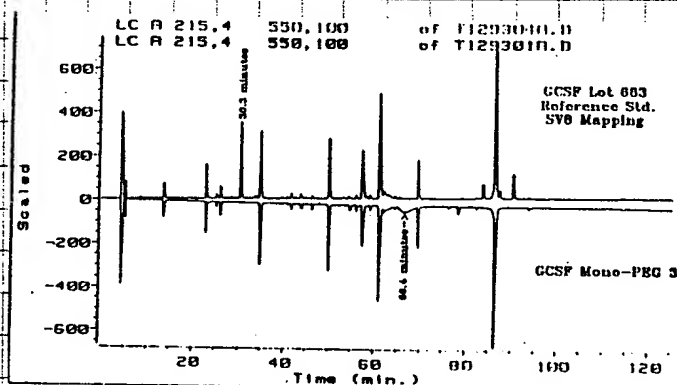
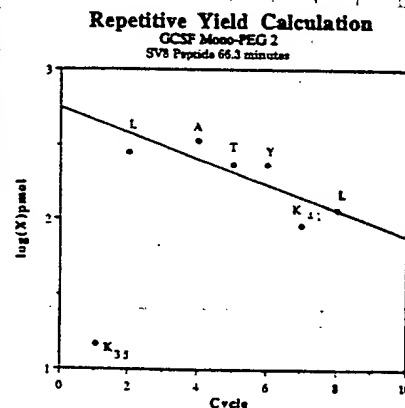
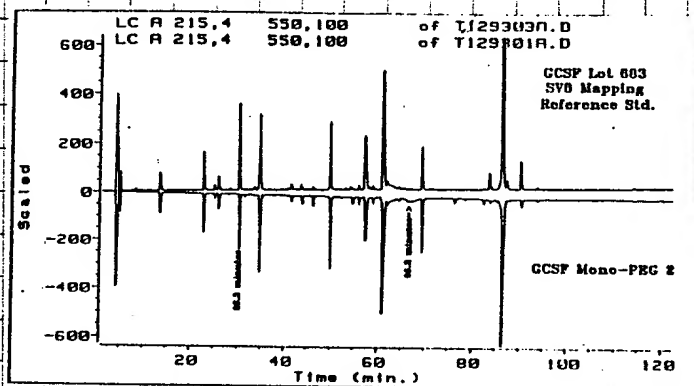
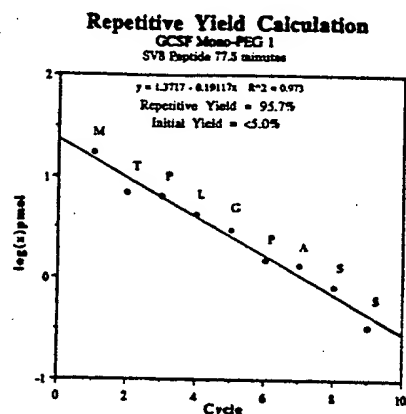
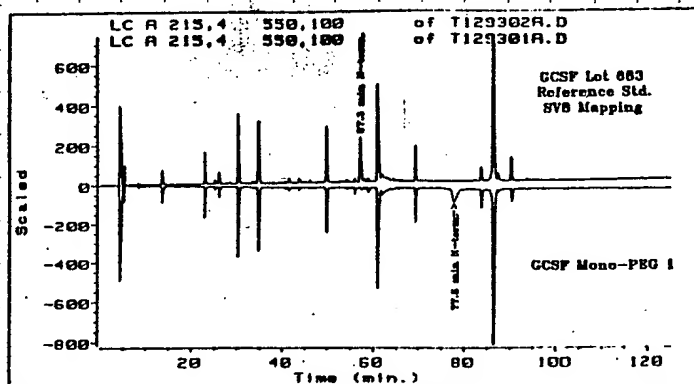
Invented by

Date _____

Recorded by

From Page No 20

REDACTED

To Page No 28

Witnessed & Understood by me,

Anne H. Grae

Date

Invented by

Christine Farnas

Recorded by

Date

From Page No. X

Materials: MONO PEG-GCSF PEAK 1A Species (from pages 1-21)
MONO PEG-GCSF PEAK 2A Species (from pages 1-21)
MONO PEG-GCSF PEAK 3A Species (from pages 1-21)
GCSF lot #T6702

50ml Amicon stirred cell
46mm YM10 Amicon membrane (X4)

REDACTED

10mM NaOAc 5% Mannitol, .004% Tween 80 pH 4.0 (made w/WFI)
(GCSF formulation buffer)

.2um CA Costar filter unit (X3)

.2um CA Nalgene filter unit

3ml sterile injection vials (X81)

13mm sterile rubber septa (X92)

13mm flip top crimp seals (X92)

5ml sterile injection vials (X11)

To Page No. 23

Witnessed & Understood by me, *

Date

Invented by

Date

Anne H. EganChristine Farnan

Recorded by

TITLE

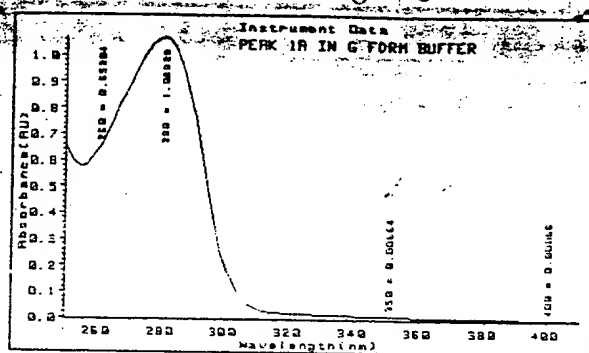
From Page No. 22

REDACTED

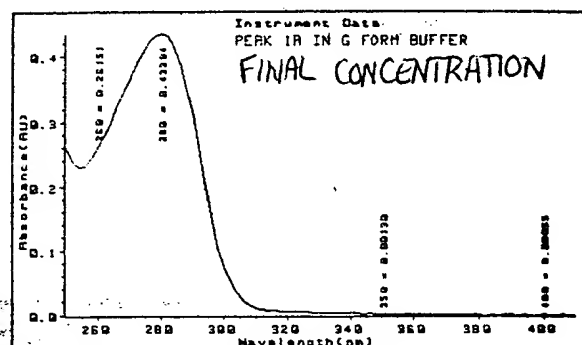
Procedure:

Peak 1A of the MONO PEG-GCSF Species

96ml of Peak 1A from page 12 was concentrated to 25ml using a YM10 46mm membrane in an Amicon stir cell. It was then buffer exchanged into GCSF formulation buffer via a pressurized reservoir connected to the stir cell. 6 retentate volume (~150ml) were exchanged. Peak 1A was then removed from the stir cell and its volume was measured @ 35.48ml by weight. An aliquot was removed and an A^{280} reading was taken:

GRAPHICSD
(LABEL / HARDCOPY)

Values : L260=0.65204 L280=1.06929 L350=0.00664 L400=0.00066
(Stddev) : (0.00063) (0.0017) (0.00022) (0.00023)

GRAPHICSD
(LABEL / HARDCOPY)

Values : L260=0.26151 L280=0.43394 L350=0.00130 L400=0.00055
(Stddev) : (0.00028) (0.00054) (0.00018) (0.00021)

$$(1.06929^{A_{280}} - 0.00104^{A_{350}}) / 0.86 = 1.236 \text{ mg/ml}$$

$$(1.236 \text{ mg/ml}) (35.48 \text{ ml}) = 6 \text{ mg} \times 0.5 \text{ mg/ml}$$

$$x = 87.71 \text{ ml} - 35.48 \text{ ml} = 52.23 \text{ ml of buffer to add}$$

$$(0.43394^{A_{280}} - 0.00130^{A_{350}}) / 0.86 = 0.503 \text{ mg/ml}$$

52.23 ml of GCSF Formulation buffer were added to the Peak 1A solution. An aliquot was taken and the A^{280} reading was rechecked. At this concentration (0.503 mg/ml) Peak 1A was sterile filtered through a 2um CA Nalgene vacuum filter unit. This solution was aliquoted into 3 ml injection vials @ 2 ml / vial. A total of 41 vials were filled. The vials were sealed with sterile septa and then crimped with aluminum crimp caps (blue). All work (filling and septa sealing) was carried out in a laminar flow hood. Vials were labeled

Mono-Peg-GCSF #6951-23
2.0ml 0.5 mg/ml

To Page No. 24

Witnessed & Understood by me,

Date

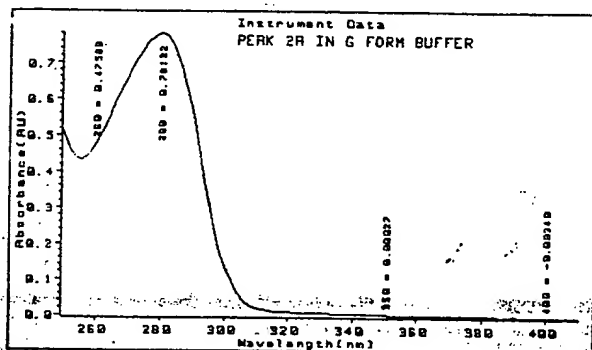
Invented by

Date

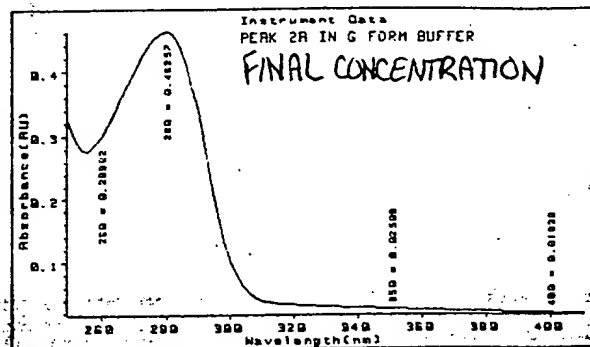
Recorded by

From Page No. 23Peak 2A of the MONO PEG-GCSF Species

48ml of Peak 2A from page 13 was concentrated to 25ml using a YM10 45mm membrane in an Amicon stir cell. It was then buffer exchanged into GCSF formulation buffer via a pressurized reservoir connected to the stir cell. 6 replicate volumes (415ul) were exchanged. Peak 2A was then removed from the stir cell and its volume was measured @ 30.75ml by weight. An aliquot was removed and an A_{280} reading was taken:

GRAPHICSD
(LABEL / HARD COPY)

Values : L260=0.47588 L280=0.78122 L350=0.00027 L400=0.00340
(Stdev) : (0.00048) (0.0012) (0.00021) (0.00020)

GRAPHICSD
(LABEL / HARD COPY)

Values : L260=0.29982 L280=0.46257 L350=0.02509 L400=0.01938
(Stdev) : (0.00028) (0.00063) (0.00019) (0.00022)

$$\begin{aligned} (0.78122^{A_{280}} / 0.0027^{A_{300}}) / 1.86 &= 0.908 \text{ mg/ml} \\ (0.908 \text{ mg/ml}) \times (30.75 \text{ ml}) &= (x \text{ ml}) \times (5 \text{ mg/ml}) \\ x &= 55.848 \text{ ml} - 30.75 \text{ ml} = 25.10 \text{ ml of buffer to add} \end{aligned}$$

$$(0.46257^{A_{280}} / 0.02509^{A_{300}}) / 1.86 = 0.508 \text{ mg/ml}$$

25.10ml of GCSF Formulation buffer was added to the Peak 2A solution. A aliquot was taken & the A_{280} reading was rechecked. At this concentration (508mg/ml) Peak 2A was sterile filtered through a 2um Corcor (A filter unit). This solution was aliquoted into 3ml injection vials @ 2ml/vial. A total of 24 vials were filled. The vials were sealed with sterile septa and then crimped sealed with aluminum crimp caps (red). All work (filling and septa sealing) was carried out in a laminar flow hood. Vials were labeled

Mono-Peg-GCSF #6931-24
2.0ml 0.5 mg/ml

To Page No. 25

Witnessed & Understood by me,

Anne H. Gar

Date

Invented by

Christine Farnan

Date

Recorded by

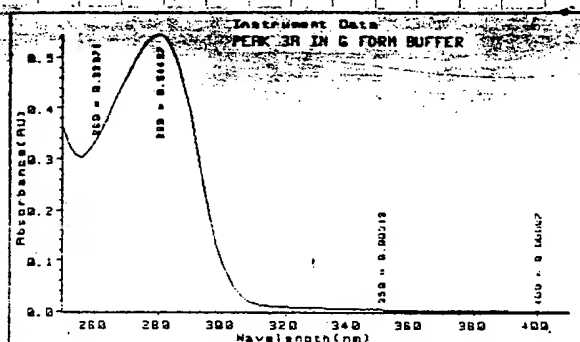
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From Page No. 24

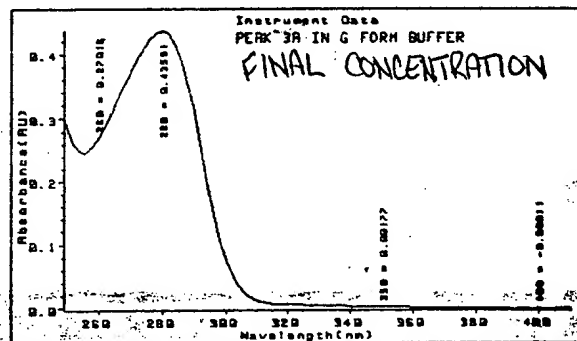
REDACTED

Peak 3A of the MONO PEG-GCSF Species

34ml of Peak 3A from page 13 was concentrated to 25ml using a YM10 43mm membrane in an Amicon stir cell. It was then buffer exchanged into GCSF Formulation buffer via a pressurized reservoir connected to the stir cell. 6 tentated volumes (~150ml) were exchanged. Peak 3A was then removed from the stir cell and its volume was measured @ 28.85ml by weight. An aliquot was removed and an A^{280} reading was taken.

GRAPHICS
(LABEL / HARD COPY)

Values : L260=0.33271 L280=0.54427 L350=0.00313 L400=0.00087
(Stdev) : (0.00032) (0.00069) (0.00021) (0.00022)

GRAPHICS
(LABEL / HARD COPY)

Values : L260=0.27816 L280=0.43591 L350=0.00177 L400=0.00011
(Stdev) : (0.00032) (0.00071) (0.00018) (0.00020)

$$(0.54427)^{A^{280}} / 0.00313^{A^{350}} / 1.86 = 1.6292 \text{ mg/ml}$$

$$(1.6292 \text{ mg/ml}) (28.85 \text{ ml}) = 6 \text{ ml} (0.5 \text{ mg/ml})$$

$$x = 36.3 \text{ ml} - 28.85 \text{ ml} = 7.4 \text{ ml of buffer to add}$$

$$(0.4391)^{A^{280}} / 0.00177^{A^{350}} / 1.86 = 0.504 \text{ mg/ml}$$

7.4ml of GCSF Formulation buffer was added to the Peak 3A solution. An aliquot was taken & the A^{280} reading was rechecked. At this concentration (0.504 mg/ml) Peak 3A was sterile filtered through a 2um CA Costar filter unit. This solution was aliquoted into 3ml injection vials @ 2ml/vial. A total of 15 vials were filled. The vials were sealed with sterile septa and then crimped sealed with aluminum crimp caps (green). All work (filling and septa sealing) was carried out in a laminar flow hood. Vials were labeled:

Mono-Peg-GCSF #6951-25
2.0ml 0.5 mg/ml

To Page No. 26

Witnessed & Understood by me,

Date

Invented by

Date

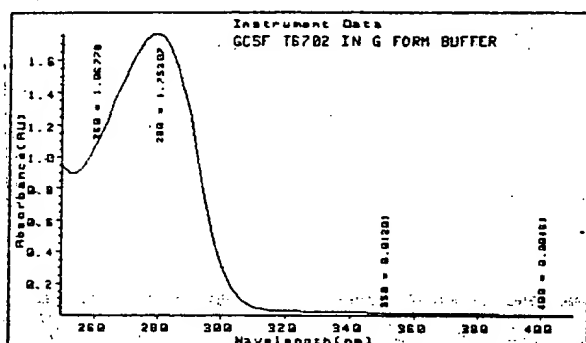
Recorded by

From Page No. 20

REDACTED

Unmodified GCSF lot #T6702

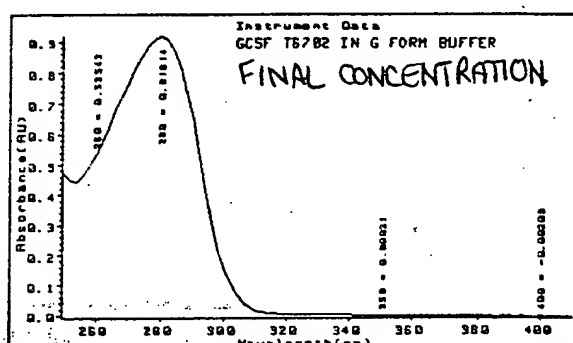
60ml of GCSF lot #T6702 were concentrated to 25ml using a YM10 43mm membrane in an Amicon stirred cell. It was then buffer exchanged into GCSF formulation buffer via a pressurized reservoir connected to the stirred cell. 6 replicate volumes (~150ml) were exchanged. The GCSF was then removed from the stirred cell and its volume was measured @ 31.75ml by weight. An aliquot was removed and an A_{280} reading was taken:



GRAPHICS

(LABEL / HARD COPY)

Values: L260=1.06779 L280=1.75307 L350=0.01201 L400=0.00461
(Stdev): (0.0015) (0.011) (0.00020) (0.00019)



GRAPHICS

(LABEL / HARD COPY)

Values: L260=0.53543 L280=0.91814 L350=0.00031 L400=0.00020
(Stdev): (0.00045) (0.0021) (0.00023) (0.00017)

$$(1.75307 \text{ AU}_{280} - 0.01201 \text{ AU}_{350}) / 0.86 = 2.0279 \text{ mg/ml}$$

$$(2.0279 \text{ mg/ml})(31.75 \text{ ml}) = x \text{ ml (1 mg/ml)}$$

$$x = 64.135 \text{ ml} - 31.75 \text{ ml} = 32.385 \text{ ml of buffer to add}$$

$$(0.91814 \text{ AU}_{280} - 0.00031 \text{ AU}_{350}) / 0.86 = 1.06 \text{ mg/ml}$$

32.385ml of GCSF formulation buffer was added to the GCSF lot #T6702 solution. An aliquot was taken and the A_{280} reading was rechecked. At this concentration (1.06 mg/ml) the GCSF lot #T6702 was sterile filtered through a 0.2um Costar CA filter unit. This solution was aliquoted into 5ml injection vials @ 5ml/vial. A total of 11 vials were filled. The vials were sealed with sterile septa and then crimped sealed with aluminum crimp caps (purple). All work (filling + septa sealing) was carried out in a biosafety flow hood. Vials were labeled and stored @ 4°C.

nu-r-GCSF lot T6702
1.0 mg/mL

To Page No. 27

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Jannan

Date

Recorded by

TITLE

From Page No. 26

LAL ANALYSIS

Aliquots were taken of the formulated 3 peaks, GCSF (+ GCSF buffer) that had been diluted down to (1 mg/mL) for the murine bioactivity study #6021593. They were placed in sterile micro tubes and submitted for LAL analysis.

To: Eva Tripp or Jill Ballog and Mashitah Waring (B1 Rm 58)
From: Elise Gabriel 8-1-A-215 x3221
Subject: Samples for LAL Analysis
Date:

Enclosed in the box are three samples of PEG-rh-GCSF, one GCSF control and one vehicle sample for LAL analysis. There is at least 1.5 mL of each. They contain 0.1 mg/mL protein and are all in the same buffer, 10 mM NaOAc, 5% mannitol, 0.004% Tween, pH 4. Please use the lot numbers printed on the labels for sample identification. Thank you.

Sample Lot No.

1. 6951-23, peak 1
2. 6951-24, peak 2
3. 6951-25, peak 3
4. GCSF lot T6702
5. Vehicle

QUALITY CONTROL ASSAY REPORT				
Product	GCSF	Spec. No.	NA	
Submitting Dept. No.	399	Product Cat.	in process	Lot No.
Method No.	A101128	Results	Endotoxin Conc.	Time
			0.24 EulmL < X < 0.48 EulmL	6.596 p.9
Analyst	Mashitah Waring	Date		Total Time
Checked By	Jill Ballog	Date		Other Changes
Notes				

QUALITY CONTROL ASSAY REPORT				
Product	Vehicle	Spec. No.	N/A	
Submitting Dept. No.	399	Product Cat.	in process	Lot No.
Method No.	A101128	Results	Endotoxin Conc.	Time
			0.12 EulmL < X < 0.24 EulmL	6.596 p.9
Analyst	Mashitah Waring	Date		Total Time
Checked By	Jill Ballog	Date		Other Changes
Notes				

QUALITY CONTROL ASSAY REPORT				
Product	PEG-rh-GCSF	Spec. No.	NA	
Submitting Dept. No.	399	Product Cat.	in process	Lot No.
Method No.	A101128	Results	Endotoxin Conc.	Time
			6951-23 peak #1: 0.12 EulmL < X < 0.24 EulmL	6.596 p.9
			6951-24 peak #2: 0.12 EulmL < X < 0.24 EulmL	6.596 p.9
			6951-25 peak #3: 0.12 EulmL < X < 0.24 EulmL	6.596 p.9
Analyst	Mashitah Waring	Date		Total Time
Checked By	Jill Ballog	Date		Other Changes
Notes				

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Christine Fauran
Recorded by

Project No. 102003Book No. (95)

TITLE _____

REDACTED

From Page No. 21MASS SPEC ANALYSIS

3 Vials Aliquots were taken of the formulated 3 peaks, G-CSF and given to Eric Watson in Analytic Chemistry for mass spec analysis.

To: Eric Watson
 From: Elise Gabriel
 Subject: Mass Spec. Analysis of PEG-GCSF Species
 Date:

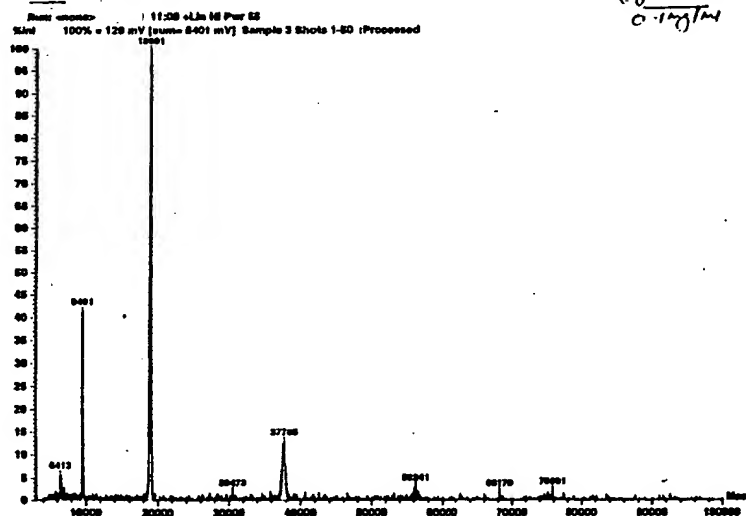
cc: Chris Farrar

You will find three species of what we believe are Mono-PEG-rh-GCSF (lot no. 6951-23, 6951-24, 6951-25) for Mass Spec. Analysis. The protein content is 0.5 mg/ml, and each vial contains 2.0 ml. The formulation buffer is 10 mM NaOAc, 5% mannitol, 0.004% Tween, pH 4.0. The molecular weight of the PEG is 6000.

We would like to confirm that these materials are indeed Mono-PEG in nature and if possible any information about the heterogeneity of the sample would be appreciated. SDS-PAGE analysis of Lot 6951-24 suggests that it might contain the most unmodified GCSF of the three lots.

If you have any questions, please call me at x3221 or Chris Farrar at x2241.

Thank You !

PEGYLATED GCSF CONTROLTo Page No. 29

Witnessed & Understood by me,

Date

Invented by

Date

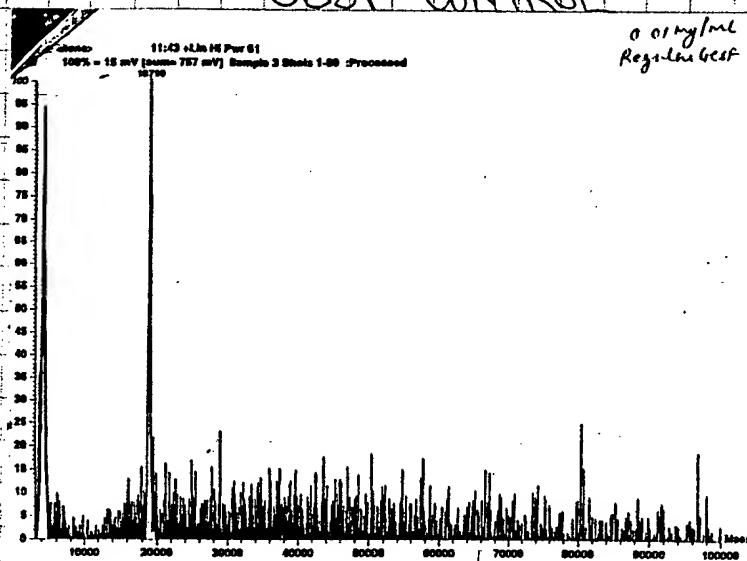
Recorded by

TITLE

From Page No. 28

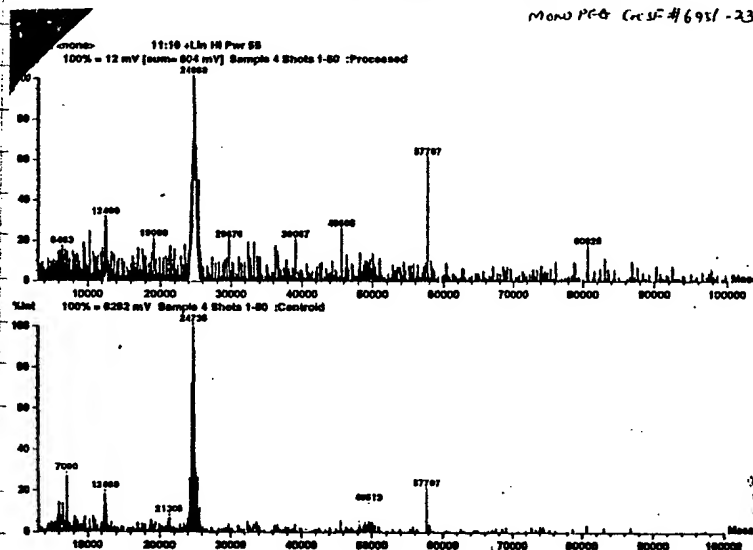
REDACTED

GCSF CONTROL



MONO PEG-GCSF #N-TERM

MONO PEG GCSF #6951-23



To Page No. 30

Witnessed & Understood by me,

Date

Invented by

Date

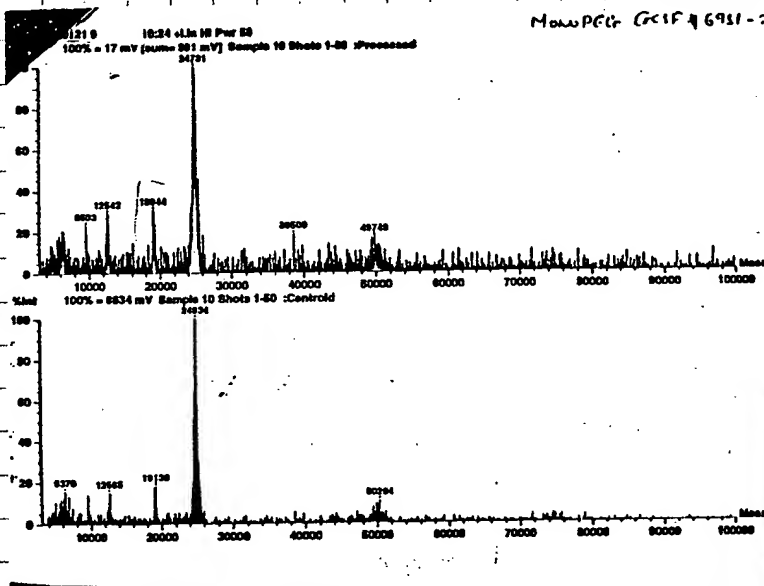
Recorded by

From Page No. 29

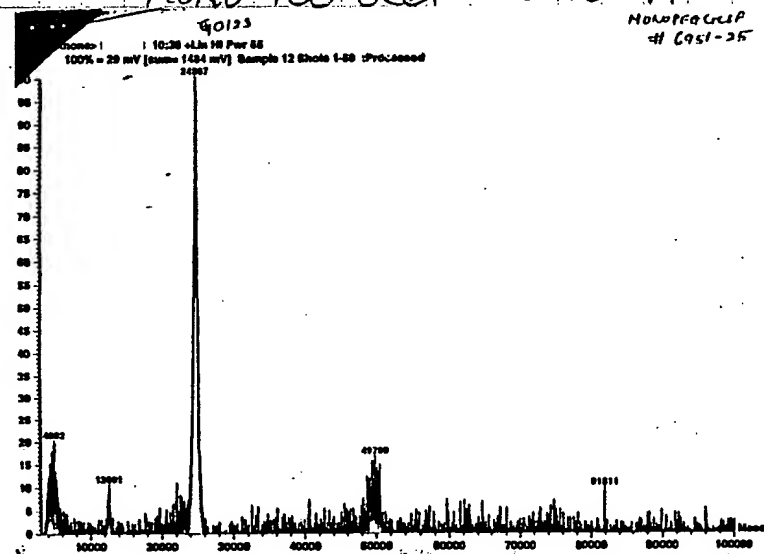
REDACTED

MONO PEG-GCSF LYS 35 (UNAP)

MONO PEG-GCSF # 6951-24



MONO PEG-GCSF LYS 41

MONO PEG-GCSF
6951-25To Page No. 31

Witnessed & Understood by me,

Anne H. Gray

Date

Invented by

Christina Fournier

Date

Recorded by

From Page No. 38IEF GEL / Horizontal

REDACTED

Ampholine[®] PAGplate

Experimental result form

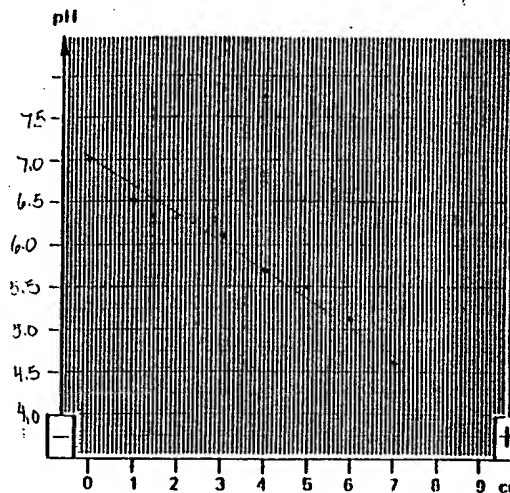
LKB

pH range 4.5-6.0Anode Electrode Solution 0.1M H₃PO₄Cathode Electrode Solution 0.1M NaOH

Date

Experiment No. Handbook No. 5575-47Operator CEE

Sample No.	Sample description	Conc. (mg/ml)	Volume (μl)	Position						
				1	2	3	4	5	6	7
1	IEF Standards 2.5-6.5	1	10	/						
2	S.P.F.	1	10	/						
3	BCSE	1	10	/						
4	MONO PEAK 1	.5	20	/						
5	" " 2	.5	20	/						
6	" " 3	.5	20	/						
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										



Electrofocusing data

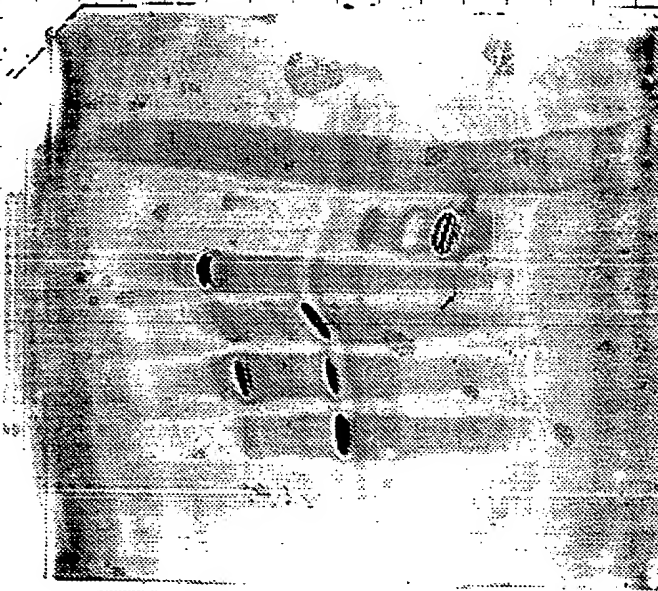
Cooling temperature

15 °C

pH measured at

4.5-6.0 °C

Time	Voltage	Current	Power
START 2:00	690 V	14.7 mA	10 W
2:30	960 V	10.4 mA	10 W
3:00	1160 V	8.7 mA	10 W
3:30	1346 V	7.4 mA	10 W
4:00	1467 V	6.9 mA	10 W
4:30	1486 V	6.7 mA	10 W
END 5:00	1515 V	6.6 mA	10 W

To Page No. 32

Witnessed & Understood by me,

Ann H. Gue

Date

Invented by

Christine J. Jansen
Recorded by

Date

From Page No. 31IEF GEL / Vertical:

REDACTED

PEG-GCSF

Date:

Operator: Chris

G-CSF gel 1

NB No: 5575-48

3-10 VERTICAL NON RED/IEF

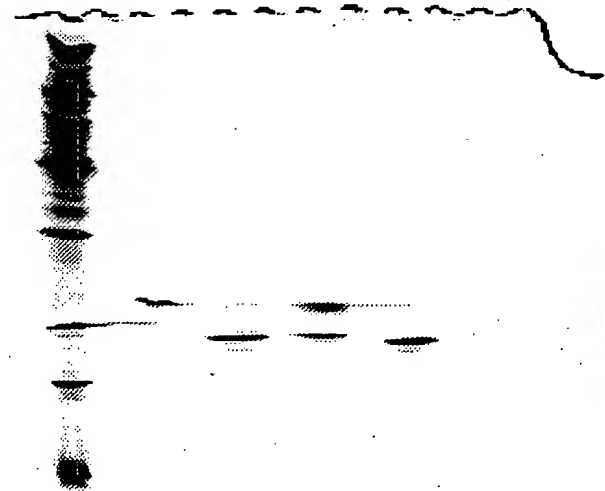
Running Conditions

constant voltage: 200:400V

all non-reduced

Comassie BIORAD Method

Sample Code	Lane No.	Conc (mg/ml)	64% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF T6702	3	1.00	0.64	7.81	5.00
mono peg-g 6951-23	5	0.50	0.32	21.88	7.00
mono peg-g 6951-24	7	0.50	0.32	21.88	7.00
mono peg-g 6951-25	9	0.50	0.32	21.88	7.00

To Page No. X

Witnessed & Understood by me,

Anne H. Gao

Date

Invented by

Christine Farnan

Date

Recorded by

From Page No. X

REDACTED

Material: 4-20% Gradient Mini Gel-SDS PAGE from I.S.S.

BioSep SEC3000 Column #41930

Coomassie G250 solutions

100mM NaPhos pH 6.9 (made w/ Milli Q)

Waters HPLC system

Microcon 10 concentrating units

EXN'S 22-25 collected & pooled from pages 2-12

EXN'S 27-30 collected & pooled from pages 2-12

1.5X Run Mixture PEG-GCSF (from NB 5576, page 83)

GCSF lot # T6702

To Page No. 34

Witnessed & Understood by me,

Ana Hoffman

Date

Invented by

Christine Fagan

Date

Recorded by

From Page No. 33

Procedure: 200ul of the pooled frn's from the two peaks were run on an HPLC SEC column and the concentration of the two species was calculated from the calibration curve. The two species were then concentrated using Microcon 10 spinners (only .5 ml aliquots). The concentrated samples were run on an SDS-PAGE Gel. The rest was stored @ 4C.

HPLC SEC:

Request # 1091

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: JW + CFProtein (Analyte): PEG-GCSFAnalysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CFColumn: SEC3000 # 41930Method: 3011

Date Results Reported: _____

Instrument # 2

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	10	55-2550	1	STD	25				
2	15	2551	1	GCSF	26				
3	.30	2552	1	"	27				
4	1.0	2553	1	"	28				
5	1.5	2554	~2	01113-16 RYN MIX	29				
6	3.4	2555	.84	01113-16 (1)	30				
7	11	2556	2.7	01113-16 (2)	31				
8	10	2557	3	1222-8	32				
9	10	2558	3	1222-8	33				
10	200	2559	7	DIPEG-GCSF	34				
11	202	2560	7	PEG-PEG-GCSF	35				
12	15	2561	2	1.5X RYN MIX	36				
13	30	2562	1	GCSF	37				
14	10	2563	1	STD	38				
15					39				
16					40				
17					41				
18					42				
19					43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 35

Witnessed & Understood by me,

Anne H. Gao

Date

Invented by

Christine Farnon

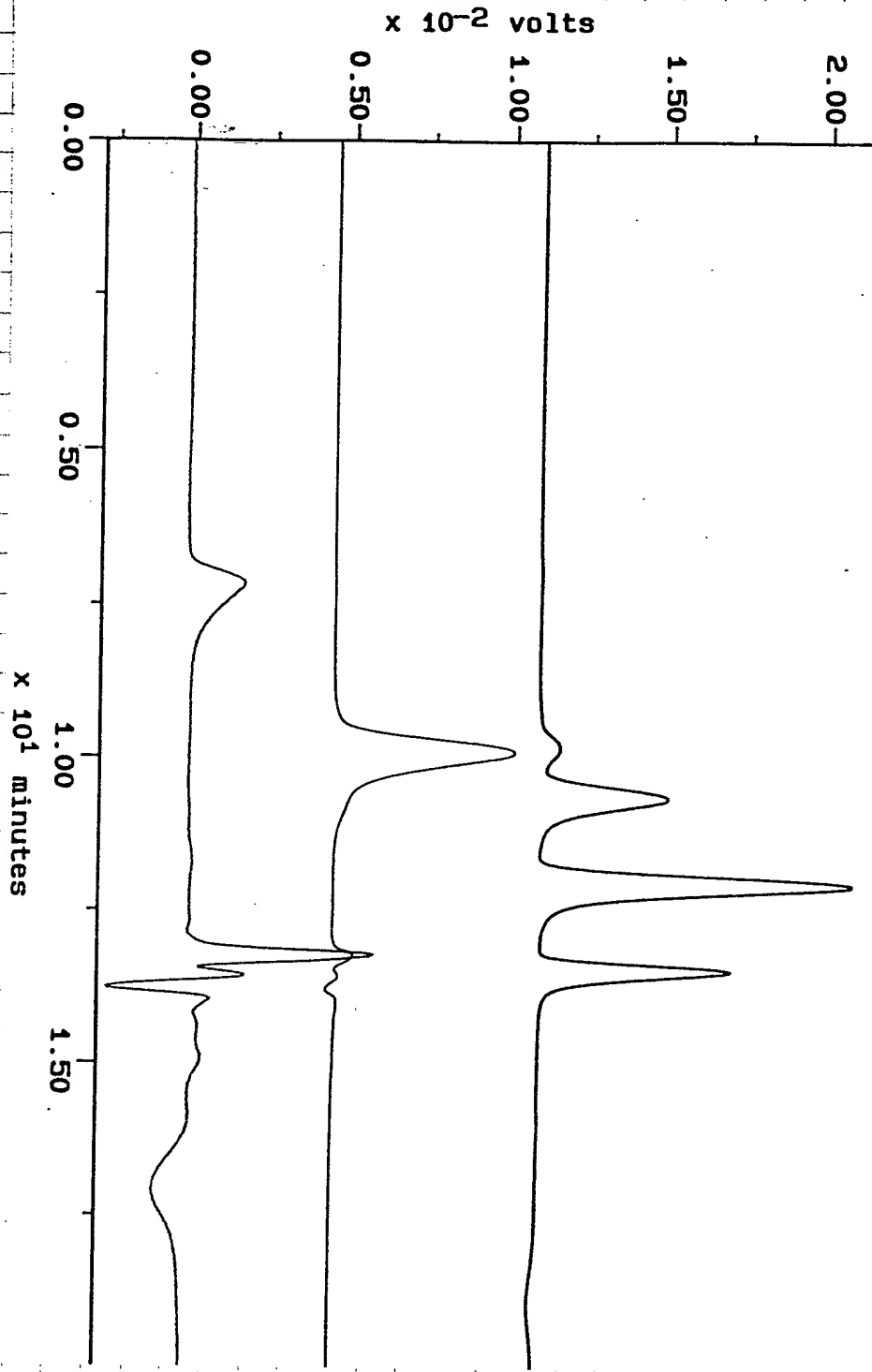
Date

Recorded by

TITLE _____

From Page No. 34

REDACTED



To Page No. 36

Witnessed & Understood by me,
Anne H. Gier

Date

Invented by
Christine Farnan
Recorded by

Date

From Page No. 30

REDACTED

SOS-PAGE:

PEG-GCSF

Date: _____

Operator: Chris

G-CSF gel 1

NB No: _____

4-20% Gradient Mini Gel

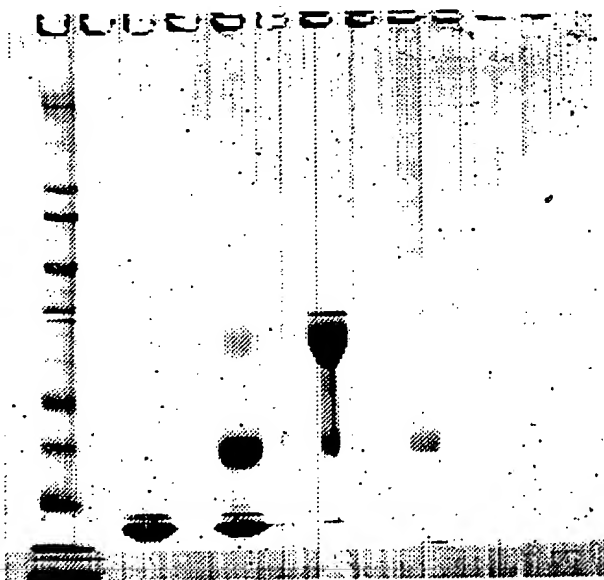
Running Conditions

constant current: 25 mA

all non-reduced

Comassie: SOP

Sample Code	Lane No.	Conc (mg/ml)	75% of Conc (mg/ml)	Load uL	Load ug
MW std	1			10.00	
GCSF	3	1.00	0.75	4.00	3.00
RXN MIX	5	2.00	1.50	6.67	10.00
DI PEG-GCSF OF SEC	7	1.48	1.11	9.01	10.00
AGGRAGATE OF SEC	9	1.66	1.25	8.03	10.00

To Page No. X

Witnessed & Understood by me,

Anne H. Gai

Date

Invented by

Christine Jasso

Date

Recorded by

From Page No. 1

REDACTED

Materials: GCSF lot #T6702

MONO PEG-GCSF lot #6951-23 (N-TERM)

MONO PEG-GCSF lot #6951-24 (LY535)

MONO PEG-GCSF lot #6951-25 (LY541)

BioSEP SEC3000 column #4958 from Phenomenex
Waters HPLC system

100mM NaPhos pH 6.9 (made w/ Milli Q)

100mM NaPhos pH 6.0 (made w/ Milli Q)

100mM NaOAc pH 5.0 (made w/ Milli Q)

100mM NaOAc pH 4.0 (made w/ Milli Q)

Purpose: To determine if depegylation is occurring for MONO-PEG-GCSF @ LY535 between the time it is loaded on the SEC column and the time it elutes from the column.

To Page No. 38

Witnessed & Understood by me,

Anne H. Gue

Date

Invented by

Christine Jones

Date

Recorded by

From Page No. 37

REDACTED

Procedure:

LY535 MONO PEG-GCSF lot #6951-24 was run on the HPLC SEC column (along w/ the controls) 4 different times. Each time using a different running buffer with a different pH ranging from 7 down to 4. Because the recovery for each of the samples decreased as the pH decreased, a comparison of the rate of recovery between the UNPEG species and the unmodified GCSF was made. Assuming that percentage of recovery should be similar for all the samples of each specific pH, the recovery of the UNPEG species seemed to decrease more rapidly than the other peaks as the pH of the running buffer lowered. See chart on page 41.

SEC HPLC

Request # 1168

Donor Submittal:	
Analytical Results Needed by: _____	
Submitted by: <u>Chris Farnas</u>	
Protein (Analyte): <u>PEG-GCSF</u>	
Analysis Requested (RP, SEC, IEX, etc.): <u>SEC</u>	
Sample Buffer Composition: _____	
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____	
Operator: <u>CF</u>	Column: <u>SEC 500 #4455</u>
Method: <u>SEC</u>	Date Results Reported: _____
Instrument # <u>2</u>	

No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification	No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification
1	20	270	1	GCSF T100	25				
2	30	270	1	GCSF T100	26				
3	60	270	1	Peak #1 #169	27				
4		270	1	Peak #2 #169	28				
5		270	1	Peak #3 #169	29				
6	30	270	1	GCSF T100	30				
7	60	270	1	Peak #1 #169	31				
8		270	1	Peak #2 #169	32				
9		270	1	Peak #3 #169	33				
10	30	270	1	GCSF T100	34				
11	60	270	1	Peak #1 #169	35				
12		270	1	Peak #2 #169	36				
13		270	1	Peak #3 #169	37				
14	30	270	1	GCSF T100	38				
15	60	270	1	Peak #1 #169	39				
16		270	1	Peak #2 #169	40				
17		270	1	Peak #3 #169	41				
18	30	270	1	GCSF T100	42				
19	60	270	1	Peak #1 #169	43				
20		270	1	Peak #2 #169	44				
21		270	1	Peak #3 #169	45				
22	30	270	1	GCSF T100	46				
23	60	270	1	Peak #1 #169	47				
24		270	1	Peak #2 #169	48				

To Page No. 39

Witnessed & Understood by me,

Date

Invented by

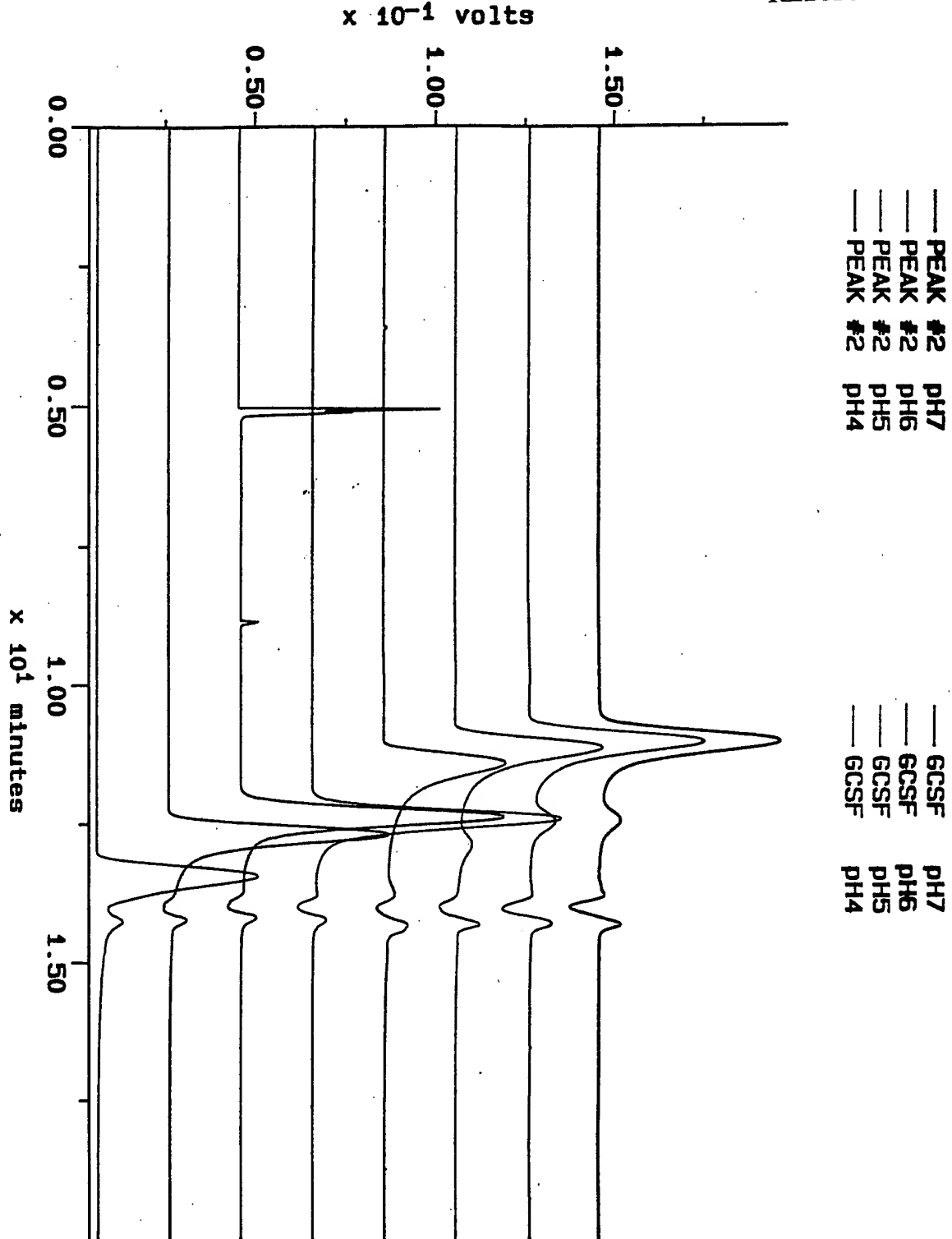
Date

Recorded by

TITLE _____

From Page No. 36

REDACTED



HPLC SEC Chromatograms @ A280

To Page No. 40

Witnessed & Understood by me,

Anne H. Gue

Date

Invented by

Christine Farrow

Recorded by

Date

From Page No. 39 HPLC SEC Peak data:

REDACTED

REQUEST: #1168 FROM: CHRIS FARRAR
SUBMITTED: REPORTED: ANALYTE: PEG-GCSF
METHOD: 3004
COLUMN: SEC3000 #41958

micrograms AREA
0.00 0
55240.9617 = SLOPE
-0.0000 = INTERCEPT
1.0000 = R SQUARED
30.00 1657229

FILE	SAMPLE	TIME	AREA	PERCENT	uL	[ANALYTE]
S3_2770	GCSF T6702 pH7	12.392	1657229 1657229	100.00	30 30	1.0000 1.0000
S3_2771	PEAK #1 pH6.9	10.967	1669629 1669629	100.00	60 60	0.5037 0.5037
S3_2772	PEAK #2 pH6.9	11.000 12.417	1488909 173071 1661980	89.59 10.41	60 60 60	0.4492 0.0522 0.5014
S3_2773	PEAK #3 pH6.9	10.925	1686139 1686139	100.00	60 60	0.5087 0.5087
S3_2774	GCSF T6702 pH6	12.383	1689881 1689881	100.00	30 30	1.0197 1.0197
S3_2775	PEAK #1 pH6.0	10.958	1597410 1597410	100.00	60 60	0.4820 0.4820
S3_2776	PEAK #2 pH6.0	11.008 12.442	1443062 189356 1632418	88.40 11.60	60 60 60	0.4354 0.0571 0.4925
S3_2777	PEAK #3 pH6.0	10.925	1631291 1631291	100.00	60 60	0.4922 0.4922
S3_2778	GCSF T6702 pH5	12.667	1514895 1514895	100.00	30 30	0.9141 0.9141
S3_2779	PEAK #1 pH5.0	11.067	1502079 1502079	100.00	60 60	0.4532 0.4532
S3_2780	PEAK #2 pH5.0	11.117 12.858	1333251 132440 1465690	90.96 9.04	60 60 60	0.4023 0.0400 0.4422
S3_2781	PEAK #3 pH5.0	11.008	1539177 1539177	100.00	60 60	0.4644 0.4644
S3_2782	GCSF T6702 pH4	13.408	1137451 1137451	100.00	30 30	0.6864 0.6864
S3_2783	PEAK #1 pH4.0	11.475	821067 821067	100.00	60 60	0.2477 0.2477
S3_2784	PEAK #2 pH4.0	11.400	1214383 1214383	100.00	60 60	0.3664 0.3664
S3_2785	PEAK #3 pH4.0	11.342	1028548 1028548	100.00	60 60	0.3103 0.3103
S3_2786	GCSF T6702 pH7	12.408	1786117 1786117	100.00	30 30	1.0778 1.0778

To Page No. 41

Witnessed & Understood by me,

Date

Invented by

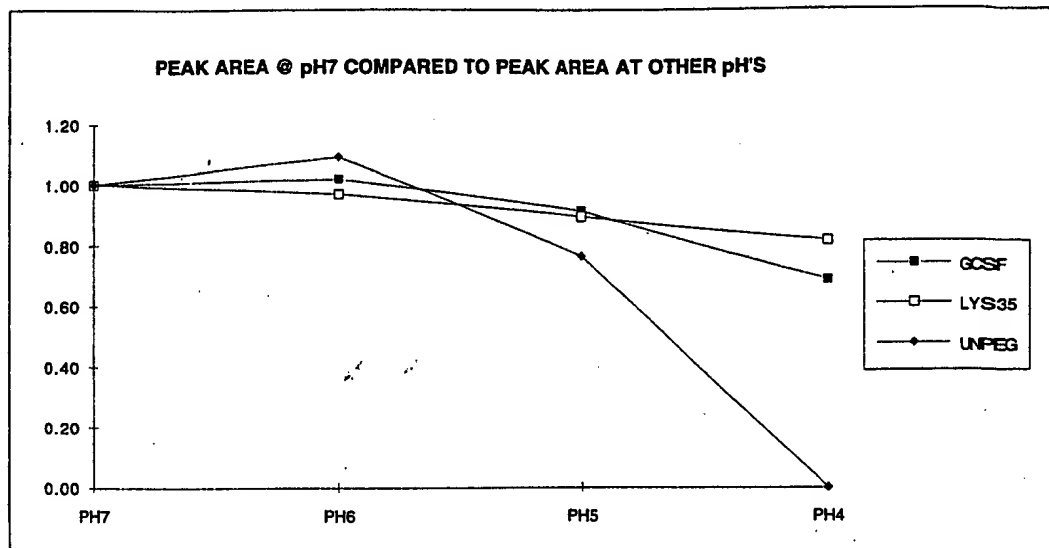
Date

Recorded by

From Page No. 40

REDACTED

	PH7	AREA	PH6	AREA	PH5	AREA	PH4	AREA
GCSF	1.00	1657229	1.02	1689881	0.91	1514895	0.89	1137451
LYS35	1.00	1488909	0.97	1443062	0.90	1333251	0.82	1214383
UNPEG	1.00	173071	1.09	189356	0.77	132440	0.00	0



To Page No. X

Witnessed & Understood by me,

Amber H. Galt

Date

Invented by

Christine Farnan

Date

Recorded by

From Page No. 2

REDACTED

PEG-GCSF LYS 35 STABILITY STUDY

PURPOSE: To study the stability of mono PEG-GCSF @ lys 35 in an elevated pH buffer 4C and at CRT, and to determine the nature of the degenerate peak.

GENERAL CONDITIONS:

LOT OF LYS 35 PEG-GCSF: 6952-24

CONC: .25mg/ml

CONTROLS: BUFFER BLANK pH4 @ CRT (10mM NaOAc, 5% Mannitol, .01% Tween 80, pH 4.0)

BUFFER BLANK pH 7 @ CRT & 4C (20mM NaPhos, 5mM NaOAc, Mannitol, .005% Tween 80, pH 6.9)

GCSF lot T6702 pH 4 @ CRT

GCSF lot T6702 pH 7 @ CRT & 4C

N-TERM PEG-GCSF pH 4 @ CRT

N-TERM PEG-GCSF pH 7 @ CRT & 4C

LYS 35 pH4 @ CRT

ASSAYS:

I) Size exclusion HPLC run on a BioSep SEC3000 column in 20mM NaPhos pH 6.9:

pH	TEMP	Time Points
7	4C	0,4,6,12,16 days
7	CRT	0,4,6,12,16 days
4 (control)	CRT	0,4,6,12,16 days
4 (control)	4C	0,16 days

Time-zero:

To Page No. 43

Witnessed & Understood by me,

Anne H. Gao

Date

Invented by

Christine J. Jansen

Date

Recorded by

111

REDACTED

Project No. 12053
Book No. 6951

4

TITLE

From Page No. 42 HPLC SEC:

Request # 1202

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: Chris Farnor
Protein (Analyte): MONO PEG-GCSE 15.35
Analysis Requested (RP, SEC, etc.): SEC
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____
Operator: CE Column: SEC3000 #49108 (used) #60242
Method: 3001 Date Results Reported: _____
Instrument # 2

Request # 1203

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: Chris Farnor
Protein (Analyte): MONO PEG-GCSE 15.35
Analysis Requested (RP, SEC, etc.): SEC
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____
Operator: CE Column: SEC3000 #60242
Method: 3001 Date Results Reported: _____
Instrument # 2

(Data pool shared by day 2)

No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification	No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification
1	10	2101	1	BLANK #101	25	120	2101	1	INTERM #101
2	10	2101	1	BLANK #101	26	120	2101	1	INTERM #101
3	10	2101	1	BLANK #101	27	120	2101	1	BLANK #101
4	10	2101	1	BLANK #101	28	120	2101	1	BLANK #101
5	10	2101	1	BLANK #101	29	120	2101	1	BLANK #101
6	10	2101	1	BLANK #101	30	120	2101	1	BLANK #101
7	10	2101	1	BLANK #101	31	120	2101	1	BLANK #101
8	10	2101	1	BLANK #101	32	120	2101	1	BLANK #101
9	10	2101	1	BLANK #101	33	120	2101	1	BLANK #101
10	10	2101	1	BLANK #101	34	120	2101	1	BLANK #101
11	10	2101	1	BLANK #101	35	120	2101	1	BLANK #101
12	10	2101	1	BLANK #101	36	120	2101	1	BLANK #101
13	10	2101	1	BLANK #101	37	120	2101	1	BLANK #101
14	10	2101	1	BLANK #101	38	120	2101	1	BLANK #101
15	10	2101	1	BLANK #101	39	120	2101	1	BLANK #101
16	10	2101	1	BLANK #101	40	120	2101	1	BLANK #101
17	10	2101	1	BLANK #101	41	120	2101	1	BLANK #101
18	10	2101	1	BLANK #101	42	120	2101	1	BLANK #101
19	10	2101	1	BLANK #101	43	120	2101	1	BLANK #101
20	10	2101	1	BLANK #101	44	120	2101	1	BLANK #101
21	10	2101	1	BLANK #101	45	120	2101	1	BLANK #101
22	10	2101	1	BLANK #101	46	120	2101	1	BLANK #101
23	10	2101	1	BLANK #101	47	120	2101	1	BLANK #101
24	10	2101	1	BLANK #101	48	120	2101	1	BLANK #101

No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification	No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification
1	10	2101	1	BLANK #101	25	120	2101	1	INTERM #101
2	10	2101	1	BLANK #101	26	120	2101	1	INTERM #101
3	10	2101	1	BLANK #101	27	120	2101	1	BLANK #101
4	10	2101	1	BLANK #101	28	120	2101	1	BLANK #101
5	10	2101	1	BLANK #101	29	120	2101	1	BLANK #101
6	10	2101	1	BLANK #101	30	120	2101	1	BLANK #101
7	10	2101	1	BLANK #101	31	120	2101	1	BLANK #101
8	10	2101	1	BLANK #101	32	120	2101	1	BLANK #101
9	10	2101	1	BLANK #101	33	120	2101	1	BLANK #101
10	10	2101	1	BLANK #101	34	120	2101	1	BLANK #101
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12	10	2101	1	BLANK #101	36	120	2101	1	BLANK #101
13	10	2101	1	BLANK #101	37	120	2101	1	BLANK #101
14	10	2101	1	BLANK #101	38	120	2101	1	BLANK #101
15	10	2101	1	BLANK #101	39	120	2101	1	BLANK #101
16	10	2101	1	BLANK #101	40	120	2101	1	BLANK #101
17	10	2101	1	BLANK #101	41	120	2101	1	BLANK #101
18	10	2101	1	BLANK #101	42	120	2101	1	BLANK #101
19	10	2101	1	BLANK #101	43	120	2101	1	BLANK #101
20	10	2101	1	BLANK #101	44	120	2101	1	BLANK #101
21	10	2101	1	BLANK #101	45	120	2101	1	BLANK #101
22	10	2101	1	BLANK #101	46	120	2101	1	BLANK #101
23	10	2101	1	BLANK #101	47	120	2101	1	BLANK #101
24	10	2101	1	BLANK #101	48	120	2101	1	BLANK #101

CONTINUATION OF STUDY FROM REQUEST #1202 (4/19/83)

1208

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: Chris Farnor
Protein (Analyte): MONO PEG-GCSE (15.35)
Analysis Requested (RP, SEC, etc.): SEC
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____
Operator: CE Column: SEC3000 #60242
Method: 3002 Date Results Reported: _____
Instrument # 2

1224

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: Chris Farnor
Protein (Analyte): MONO PEG-GCSE
Analysis Requested (RP, SEC, etc.): SEC
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____
Operator: CE Column: SEC3000 #60242
Method: 3002 Date Results Reported: _____
Instrument # 3

No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification	No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification
1	10	2101	1	BLANK #101	25	120	2101	1	INTERM #101
2	10	2101	1	BLANK #101	26	120	2101	1	INTERM #101
3	10	2101	1	BLANK #101	27	120	2101	1	BLANK #101
4	10	2101	1	BLANK #101	28	120	2101	1	BLANK #101
5	10	2101	1	BLANK #101	29	120	2101	1	BLANK #101
6	10	2101	1	BLANK #101	30	120	2101	1	BLANK #101
7	10	2101	1	BLANK #101	31	120	2101	1	BLANK #101
8	10	2101	1	BLANK #101	32	120	2101	1	BLANK #101
9	10	2101	1	BLANK #101	33	120	2101	1	BLANK #101
10	10	2101	1	BLANK #101	34	120	2101	1	BLANK #101
11	10	2101	1	BLANK #101	35	120	2101	1	BLANK #101
12	10	2101	1	BLANK #101	36	120	2101	1	BLANK #101
13	10	2101	1	BLANK #101	37	120	2101	1	BLANK #101
14	10	2101	1	BLANK #101	38	120	2101	1	BLANK #101
15	10	2101	1	BLANK #101	39	120	2101	1	BLANK #101
16	10	2101	1	BLANK #101	40	120	2101	1	BLANK #101
17	10	2101	1	BLANK #101	41	120	2101	1	BLANK #101
18	10	2101	1	BLANK #101	42	120	2101	1	BLANK #101
19	10	2101	1	BLANK #101	43	120	2101	1	BLANK #101
20	10	2101	1	BLANK #101	44	120	2101	1	BLANK #101
21	10	2101	1	BLANK #101	45	120	2101	1	BLANK #101
22	10	2101	1	BLANK #101	46	120	2101	1	BLANK #101
23	10	2101	1	BLANK #101	47	120	2101	1	BLANK #101
24	10	2101	1	BLANK #101	48	120	2101	1	BLANK #101

No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification	No.	Inj. Vol.	File Name	Conc. (mg/ml)	Sample Identification
1	10	2101	1	BLANK #101	25	120	2101	1	INTERM #101
2	10	2101	1	BLANK #101	26	120	2101	1	INTERM #101
3	10	2101	1	BLANK #101	27	120	2101	1	BLANK #101
4	10	2101	1	BLANK #101	28	120	2101	1	BLANK #101
5	10	2101	1	BLANK #101	29	120	2101	1	BLANK #101
6	10	2101	1	BLANK #101	30	120	2101	1	BLANK #101
7	10	2101	1	BLANK #101	31	120	2101	1	BLANK #101
8	10	2101	1	BLANK #101	32	120	2101	1	BLANK #101
9	10	2101	1	BLANK #101	33	120	2101	1	BLANK #101
10	10	2101	1	BLANK #101	34	120	2101	1	BLANK #101
11	10	2101	1	BLANK #101	35	120	2101	1	BLANK #101
12	10	2101	1	BLANK #101	36	120	2101	1	BLANK #101
13	10	2101	1	BLANK #101	37	120	2101	1	BLANK #101
14	10	2101	1	BLANK #101	38	120	2101	1	BLANK #101
15	10	2101	1	BLANK #101	39	120	2101	1	BLANK #101
16	10	2101	1	BLANK #101	40	120	2101	1	BLANK #101
17	10	2101	1	BLANK #101	41	120	2101	1	BLANK #101
18	10	2101	1	BLANK #101	42	120	2101	1	BLANK #101
19	10	2101	1	BLANK #101	43	120	2101	1	BLANK #101
20	10	2101	1	BLANK #101	44	120	2101	1	BLANK #101
21	10	2101	1	BLANK #101	45	120	2101	1	BLANK #101
22	10	2101	1	BLANK #101	46	120	2101	1	BLANK #101
23	10	2101	1	BLANK #101	47	120	2101	1	BLANK #101
24	10	2101	1	BLANK #101	48	120	2101	1	BLANK #101

To Page No. 44

Witnessed & Understood by me,

Date

Invented by

Date

Anne H. Gao

Christine Farnor

Recorded by

From Page No. 43

HPLC overlays:

REDACTED

HPLC SEC Chromatograms e A80 for N-Term PEG-GCSF CONTROL

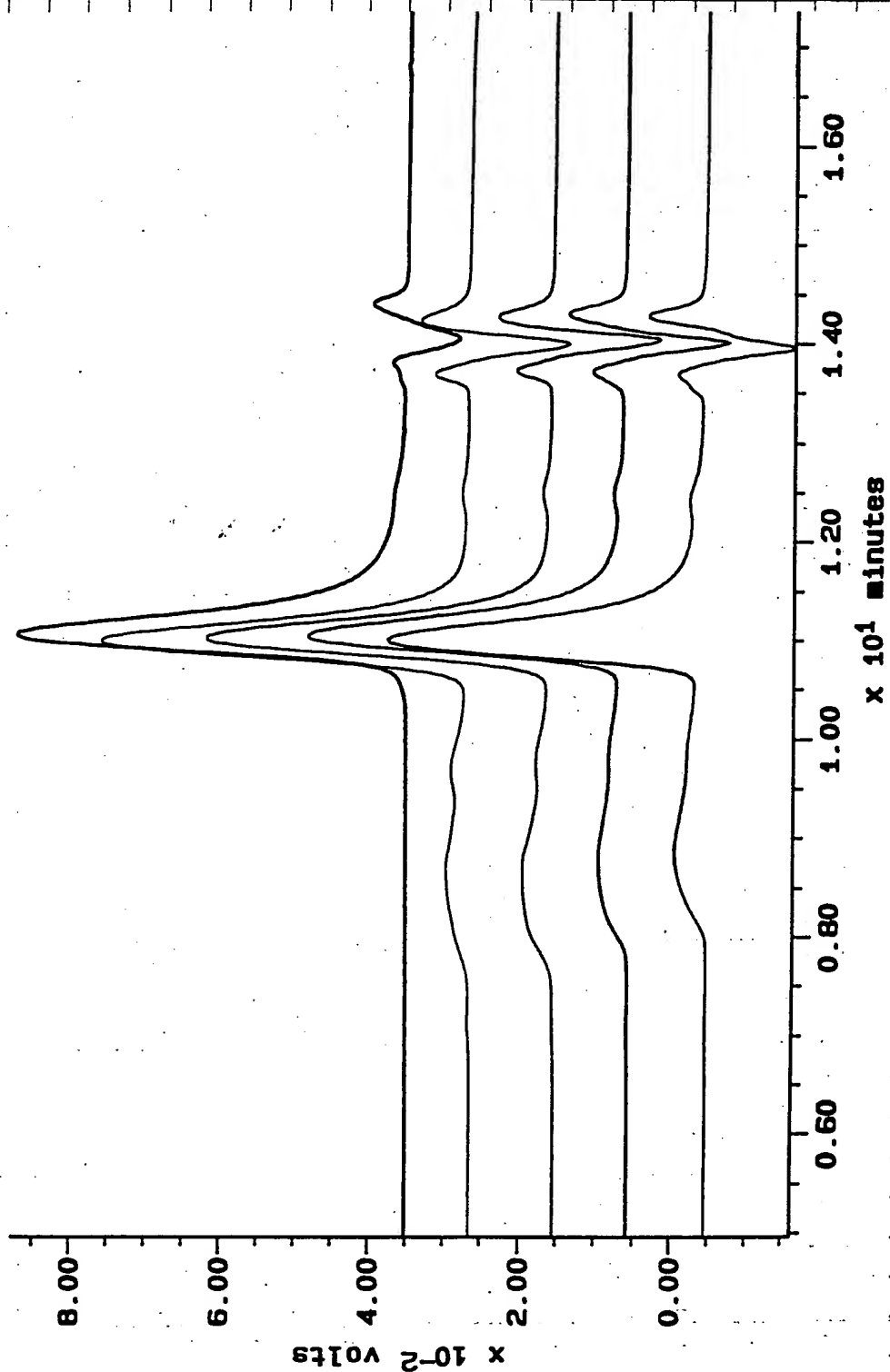
— NTERM pH6 T=16

— NTERM pH6 T=0

— NTERM pH6 T=4

— NTERM pH6 T=6

— NTERM pH6 T=12

To Page No. 45

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Farrar

Date

Recorded by

TITLE _____

REDACTED

From Page No. 44

HPLC SEC Chromatograms @ 280 nm for LYS35 PEG-GCSF + UNPEG Species

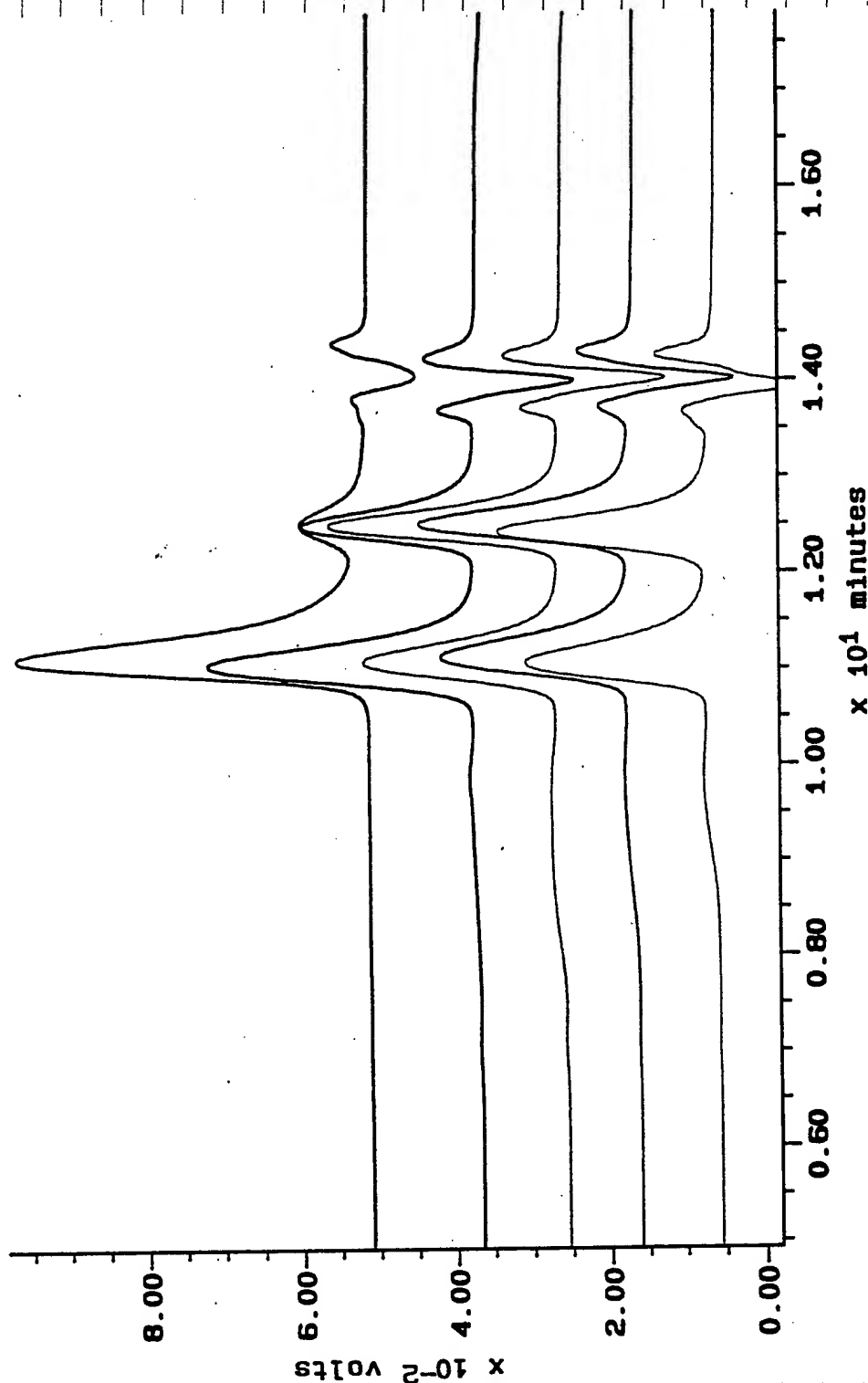
— LYS35 pH6 T=16

— LYS35 pH6 T=0

— LYS35 pH6 T=4

— LYS35 pH6 T=6

— LYS35 pH6 T=12



To Page No. 44

Witnessed & Understood by me,

Anne H. H. H.

Date

Invented by

Christine M. Jones

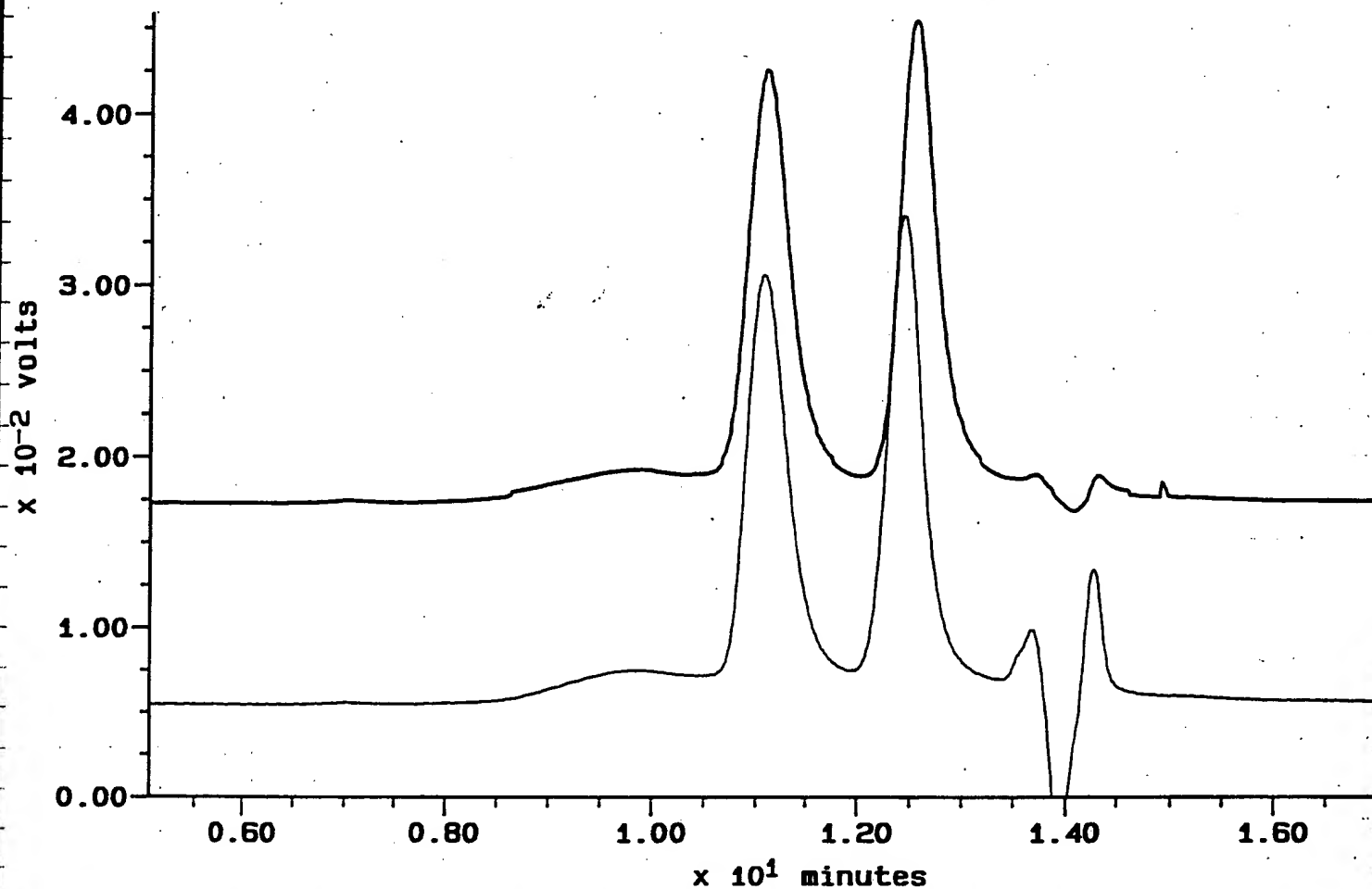
Recorded by

Date

From Page No. 45

Chromatograms @ A_{220} for Lys35 species @ 16 + 35 days stored @ elevated pH:

— LYS35 pH7 T=350
 — LYS35 pH7 T=160



Discussion:

From the chromatograms on page 45 + 46, it appears that the depegylation is driven to completion by the 16th day at the elevated pH. The proportions of the peaks don't change much after this day, even at the 35th day. The proportion of the Lys35 mono peg-GCSF peak and the UNPEG peak, after the depegylation is driven to completion, appears to be about 50:50.

Raising the temp. increased the rate of depegylation, but it increased the rate of aggregation as well. To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Annette H. Gao

Christine Fournier

Recorded by

From Page No. 46

REDACTED

Materials: PEG-GCSF lot # 6951-24 (LYS35) 3mg / $\frac{1}{6}$ vials

Superdex 75 / 24ml column # 9228096 from Pharmacia
Pharmacia FPLC system

20mM NaOAc pH 4.0 (made w/ WFI)

100mM NaPhos pH 6.9 (made w/ Milli Q)

Centricon 10 concentrating units by Amicon

4-20% Gradient Mini Gel SDS PAGE from I.S.S.

Coomassie G250 solutions

Biosep SEC 8000 column # 50242 from Phenomenex

#

Peptide mapping reagents: 8M Urea (480mg Urea + 640ul H_2O)

.5M NaBrate (in H_2O)

.8M TBP (tributyl phosphine) in DMSO (dimethyl sulfoxide)
(9ul TBP + 441 ul DMSO) fresh

.4M ABO-F (7-fluoro-4-sulfamoyl-2,1,3-benzoxadiazole)
in DMSO (ABO-F from Wako)

1M Tris-Cl pH 8.5 (in H_2O)

Milli Q H_2O

Endo Lys C (stock @ 1mg/ml in 20mM Tris pH 8.5)
L (from Wako)

Cy column # 4911 from Uydac

Reverse Phase Solvents @ .1% Trifluoro Acetic Acid (TFA) in Burdick +
Jackson High Purity Water

@ 95% Acetonitrile (CH_3CN), 5% High Purity
Water, .1% TFA

GCSF formulation buffer - 10mM NaOAc, 5% Mannitol, .004% Tween 80 pH 4.0
(made w/ WFI)

To Page No. 48

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Project No. 10093

Book No. 6961 TITLE

REDACTED

From Page No. 4

Procedure:

3 ml of PEG-GCSF lot # 6961-24 (1 ml) was added to 1 ml of 100 mM NaPhos pH 6.9 to establish an elevated pH environment. The PEG-GCSF was stored this way @ 4°C for 6 days, then it was run on an FPLC SEC column & the different components were separated.

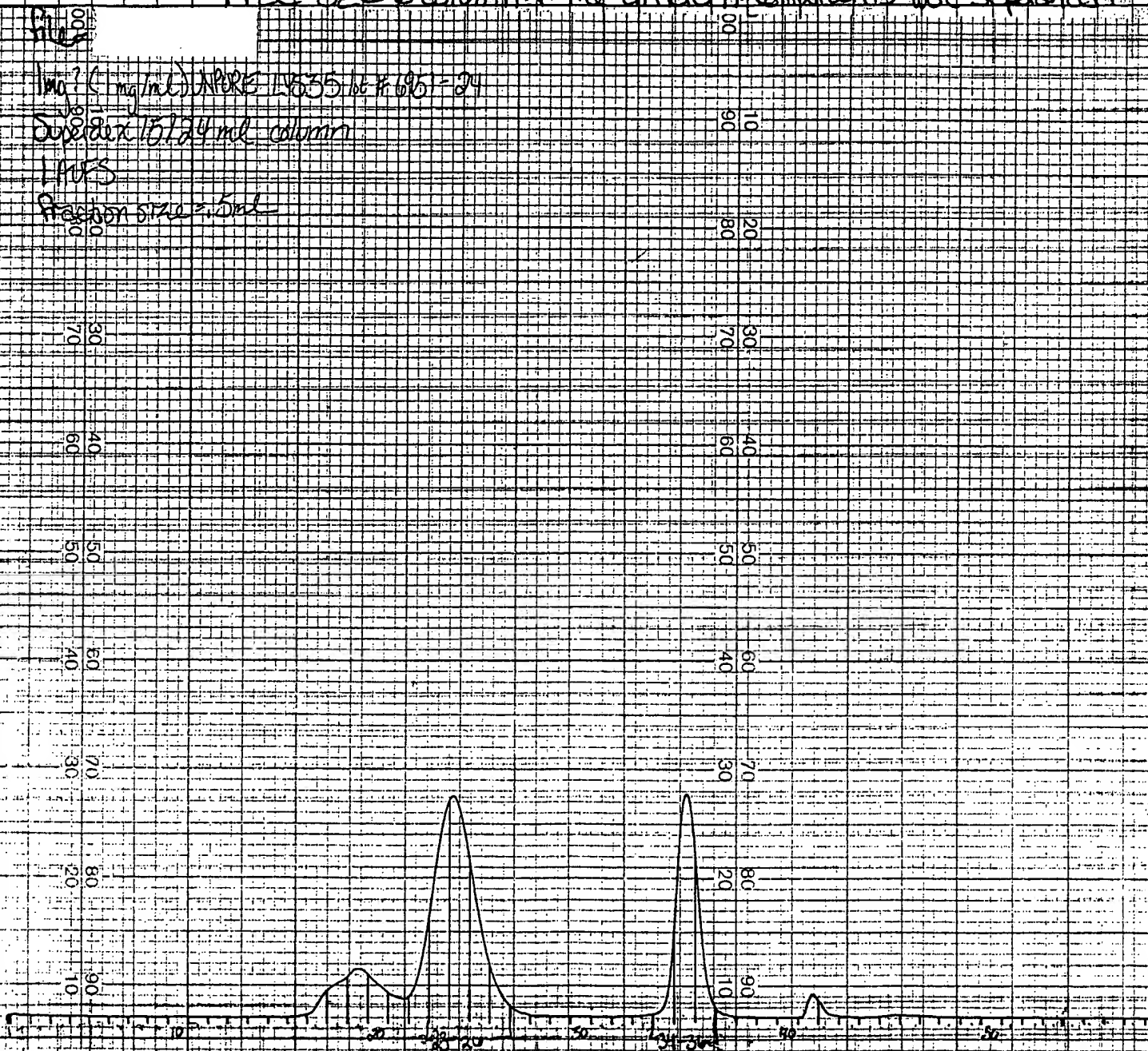
File #

1 mg? (mg/ml) APRE 1855 lot # 6961-24

Superdex 16/24 ml column

LHES

Fraction size = 5 ml



To Page No. 49

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Jones

Date

Recorded by

TITLE _____

REDACTED

From Page No. 48

FXN'S 23-26 & 34-36 were collected, pooled, and stored @ 4°C

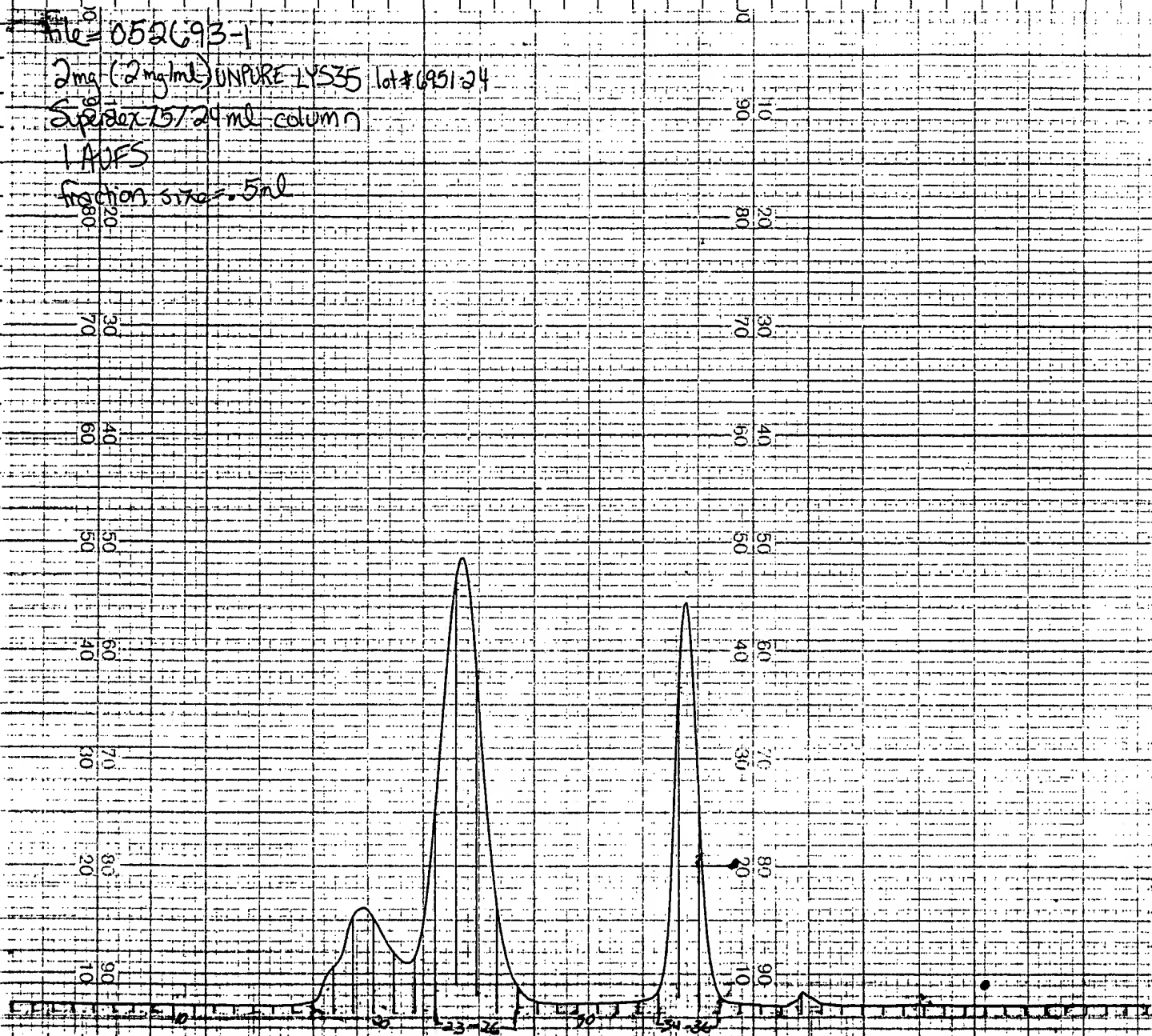
File # 052693-1

2mg (2mg/ml) UNPURE LYS35 lot # 6951-24

Superdex 15/24 ml column

1 ADFS

fraction size = .5ml



To Page No. 50

Witnessed & Understood by me,

Anne H. Gade

Date

Invented by

Christine Farrar

Recorded by

Date

From Page No. 49

FPLC Overlay

FPLC Chromatograms @ A280 of the incubated LYS35 species + GCSF

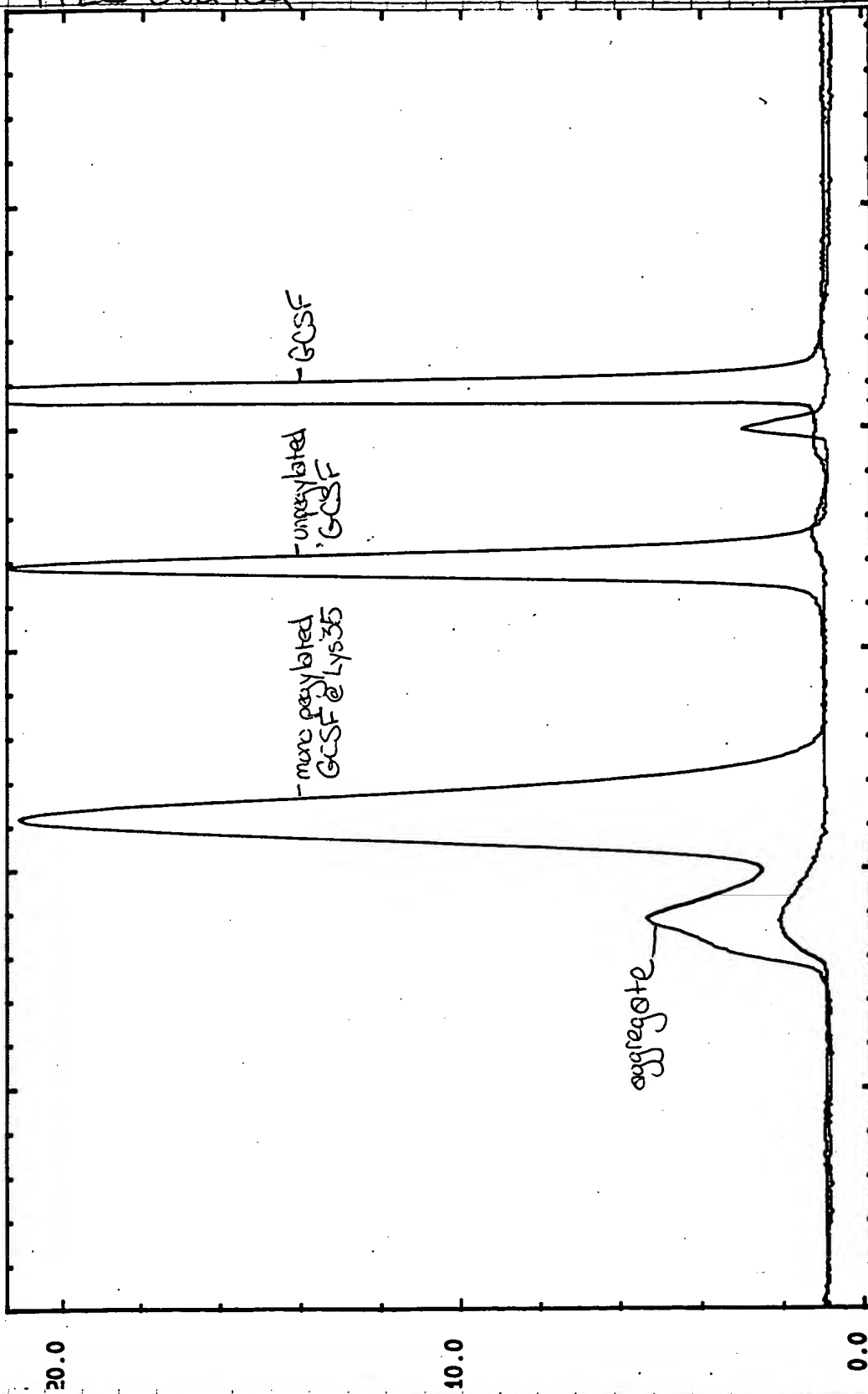
Chromatogram : 052493-4

Run note : 1MG LYS35 UNPURIFIED 1AUF5

User : FARRAR

Run date :

time : 16:19:16



min

This overlay shows the dramatic difference in hydrodynamic size between the unpeyolated form of GCSF and the unmodified form of GCSF.

To Page No. 51

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Anne H. Gar

Christine Farrar

TITLE

From Page No. 50

SOS PAGE:

REDACTED

PEG-GCSF

Date: _____

Operator: Chris

PEG-GCSF

NB No:

4-20% Gradient Mini Gel

Running Conditions

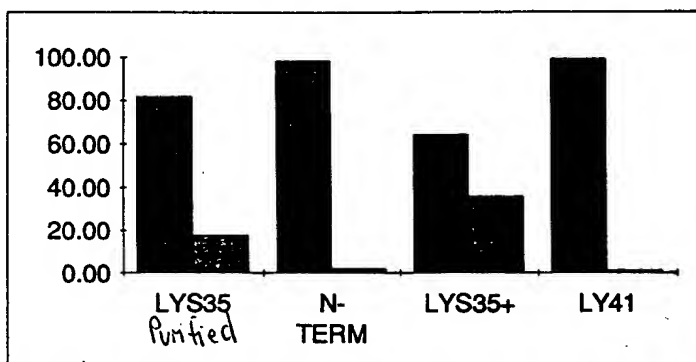
constant current: 25 mA

all non-reduced

4-20% Gradient Mini Gel/commasie SOP

Sample Code	Lane No.	Conc (ng/ml)	80% of Conc (ng/ml)	Load uL	Load ug
MW STD	1			10	
GCSF T6702	3	1.00	0.80	6	5.00
NTERM 6951-23	5	0.50	0.40	25	10.00
LYS 35 6951-24	7	0.50	0.40	25	10.00
LYS 41 6951-25	9	0.50	0.40	25	10.00
LYS 35 PURIFIED	11	0.50	0.40	25	10.00

PEAK ID	LYS35	N-TERM	LYS35+	LY41
PEG-GCSF	81.83	98.10	64.11	99.00
UNPEG-GCSF	17.41	1.90	35.89	1.00
GCSF	0.76	0.00	0.00	0.00



To Page No. 52

Witnessed & Understood by me,

Date _____

Invented by

Date

Recorded by

From Page No. 51

REDACTED

HPLC SEC

Request # 1252

Date Submitted: _____
Analytical Results Needed by: _____
Submitted by: Chris Farrar
Protein (Analyte): HONO PEG-GCSF
Analysis Requested (RP, SEC, IEX, etc.): SEC
Sample Buffer Composition: _____
Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CF Column: SEC3000 #50242
Method: 3003 Date Results Reported: _____
Instrument # 2

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol.	File Name	Conc mg/ml	Sample Identification
1	10	3103	-	STD	25				
2	15	3104	1	GCSF-T6702	26				
3	30	3105	1	"	27				
4	60	3106	1	"	28				
5	60	3107	1.5	N-TERM	29				
6		3108		LYS 58	30				
7		3109		LYS 41	31				
8	↓	3110	↓	LYS 35 Puffed	32				
9	30	3111	1	GCSF-T6702	33				
10	10	3112	-	STD	34				
11					35				
12					36				
13					37				
14					38				
15					39				
16					40				
17					41				
18					42				
19					43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 53

Witnessed & Understood by me,

Date

Invented by

Date

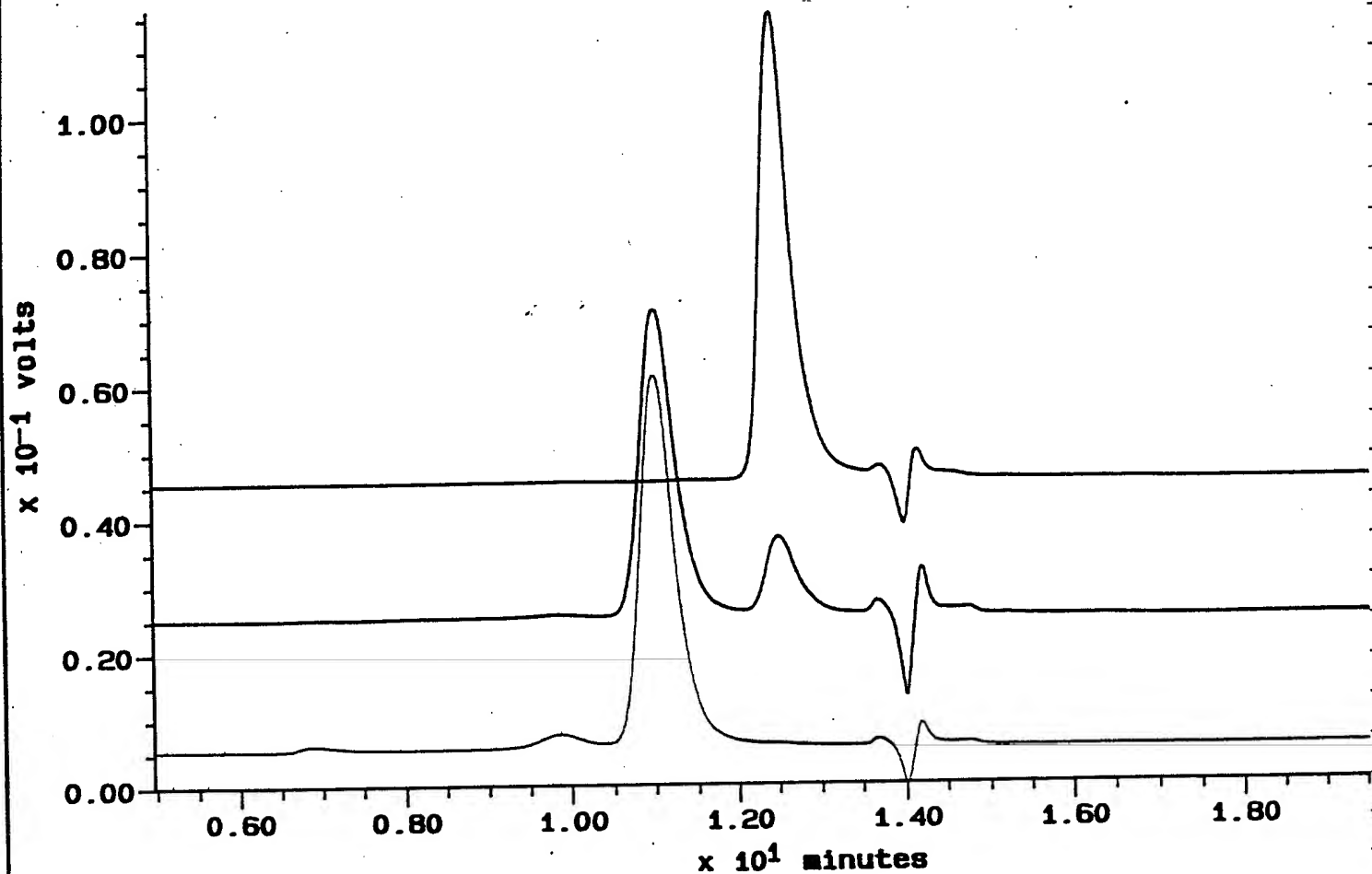
Recorded by

TITLE _____

From Page No. 52

REDACTED

— GCSF T6702
— LYS35 6951-25
— LYS35 PURIFIED



Hydrodynamic molecular wt.

Lys35 pegylated GCSF \approx 60,000

UNPEGylated GCSF \approx 35,500

GCSF \approx 18,800

To Page No. 54

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 53PEPTIDE MAPPING:

1mg aliquots of GCSF, LYS35 purified (from pages 48, 49), & UNPEG species (from pages 48, 49) were dried down in a speed-vac system.

The samples were reconstituted with 100ul of 8M Urea, 10ul of 1M Tris Cl pH 8.5, 50ul of Milli Q H₂O, and 40ul of Endo LysC reagent. The samples were incubated this way @ CRT overnight.

One at a time: To ~~each~~ ^{the} sample was added 50ul of .5M NaBorate, 12.5ul of TBP (80mM) in DMSO, 25ul of ABD-F (40uM). The sample was incubated @ 60°C for 10 minutes in a hot water bath, then it was loaded onto a C₄ column and run with the following gradient:
(This method was developed by Mike Trewhett)

Evt #	Time min	Flow ml/min	Composition %A	%B	Curve Type
1	0.0	0.7	97.2	2.8	
2	70.0	0.7	47.9	52.1	6
3	75.0	0.7	47.9	52.1	6
4	85.0	0.7	24.2	75.8	6
5	95.0	0.7	24.2	75.8	6
6	95.1	0.7	97.2	2.8	6
7	105.0	0.7	97.2	2.8	6
8	105.1	0.0	97.2	2.8	6

A = .1% TFA in H₂OB = 95% CH₃CN, 5% H₂O, .1% TFADetectors: UV @ A₂₈₀
Fluorescence

REQUEST # 1331

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: Chris FarnasProtein (Analyte): MONO PEG-GCSF @ LYS35 (LYSC MAP)Analysis Requested (RP, SEC, IEX, etc.): RP

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CE + MTColumn: C₄ Vydac #494Method: GHAP-1

Date Results Reported: _____

Instrument # 1

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	200	GHAP203	-	MP EG LYS 35	25				
2	175	285	-	GCSF TL702	26				
3	175	286	-	UNPEG GCSF	27				
4					28				
5					29				
6					30				
7					31				

To Page No. 55

Witnessed & Understood by me,

Date

Invented by

Date

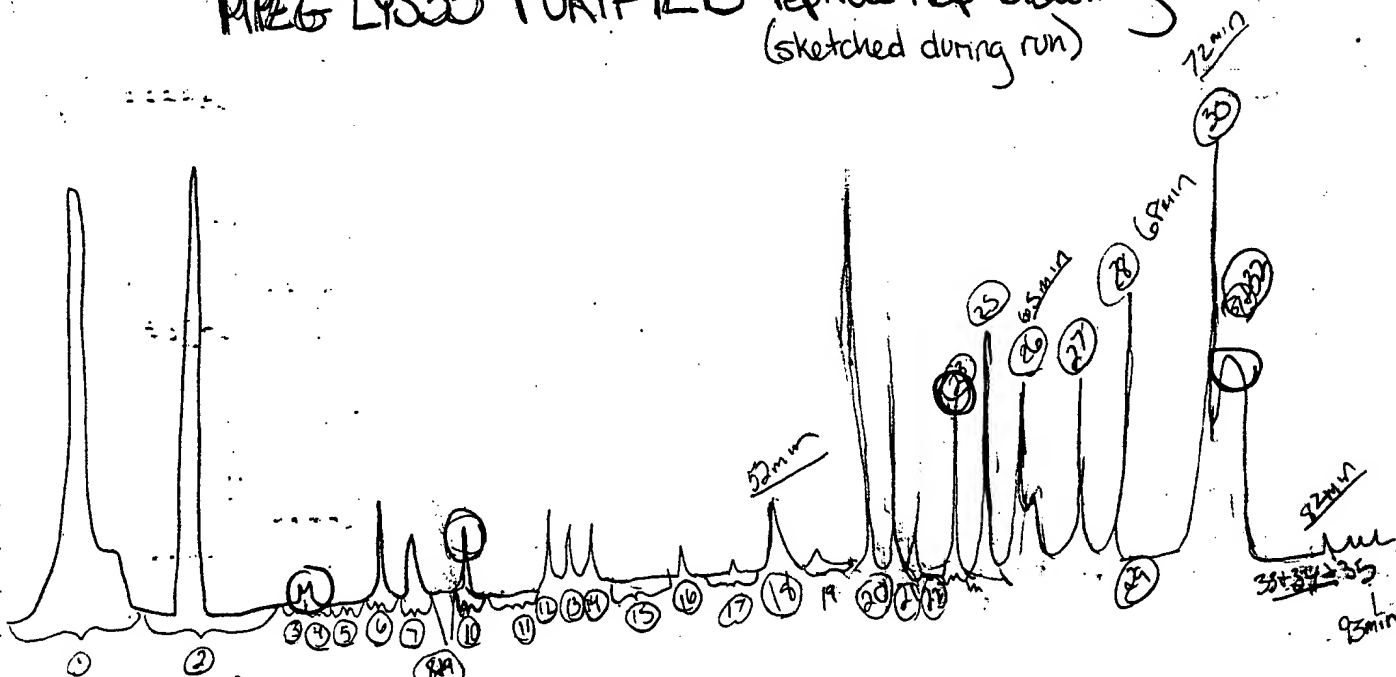
Recorded by

Anne H. GaoChristine Farnas

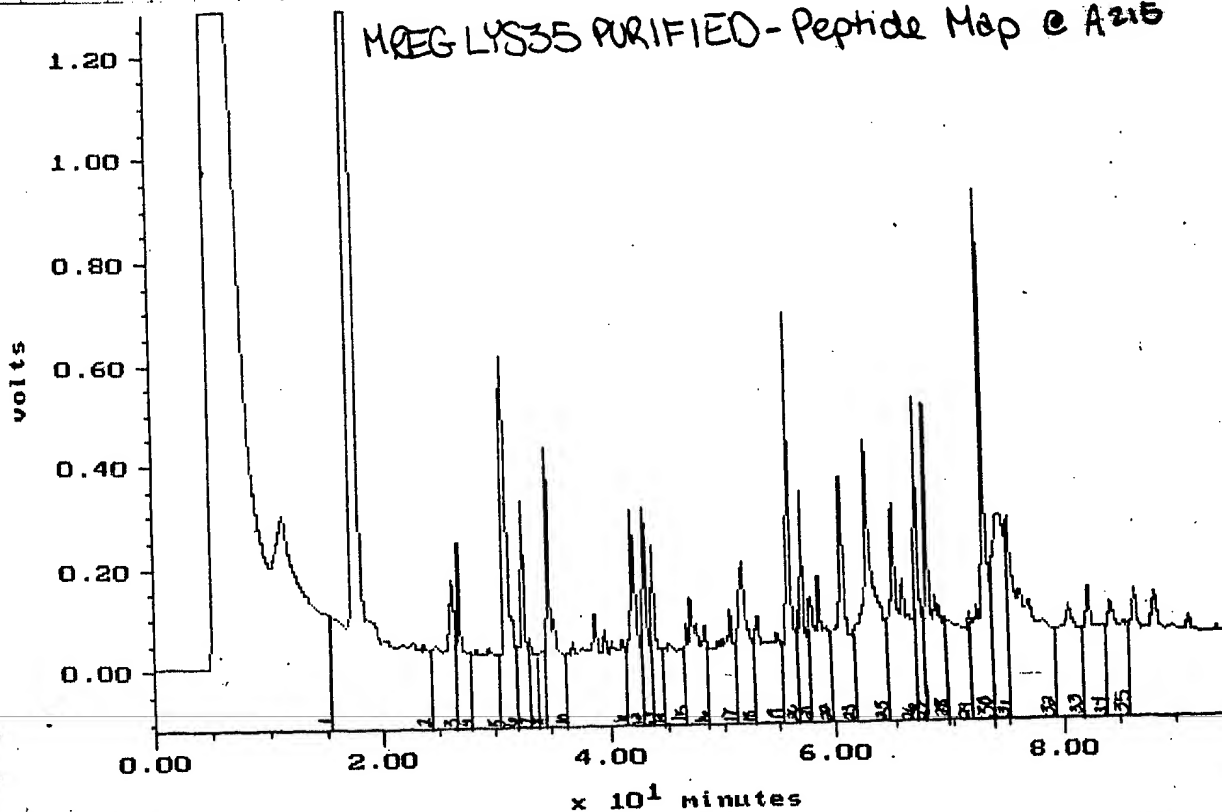
TITLE

From Page No. 54 Baseline was monitored + sketched. FYN'S were numbered + hand collected:

MREG LYS35 PURIFIED-Peptide Map Drawing (sketched during run)



MREG LYS35 PURIFIED-Peptide Map @ A215



To Page No. 56

Witnessed & Understood by me,

Amber H. Cole

Date

Invented by

Christine Foman

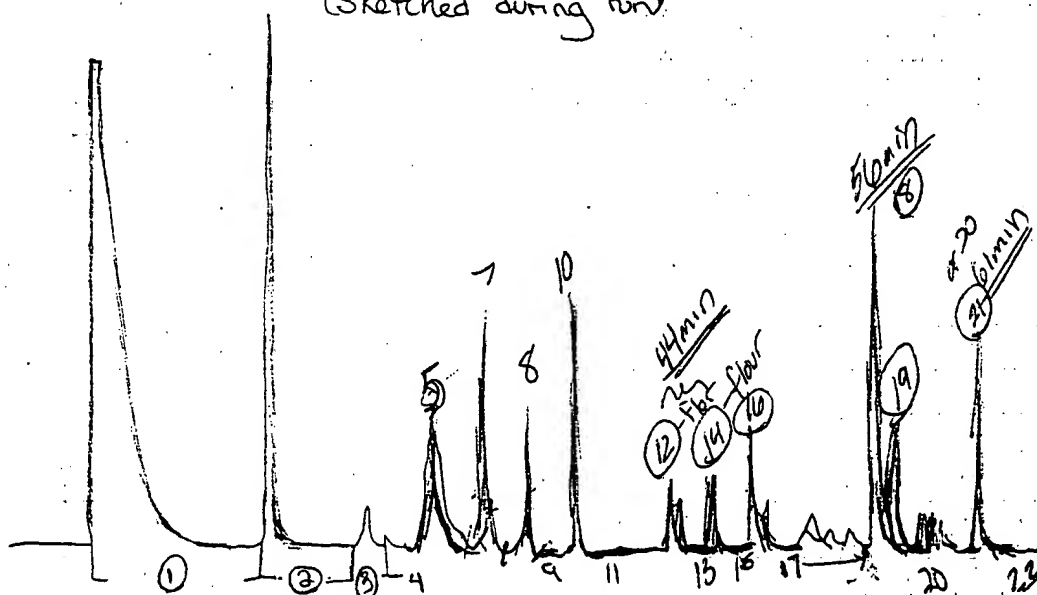
Recorded by

Date

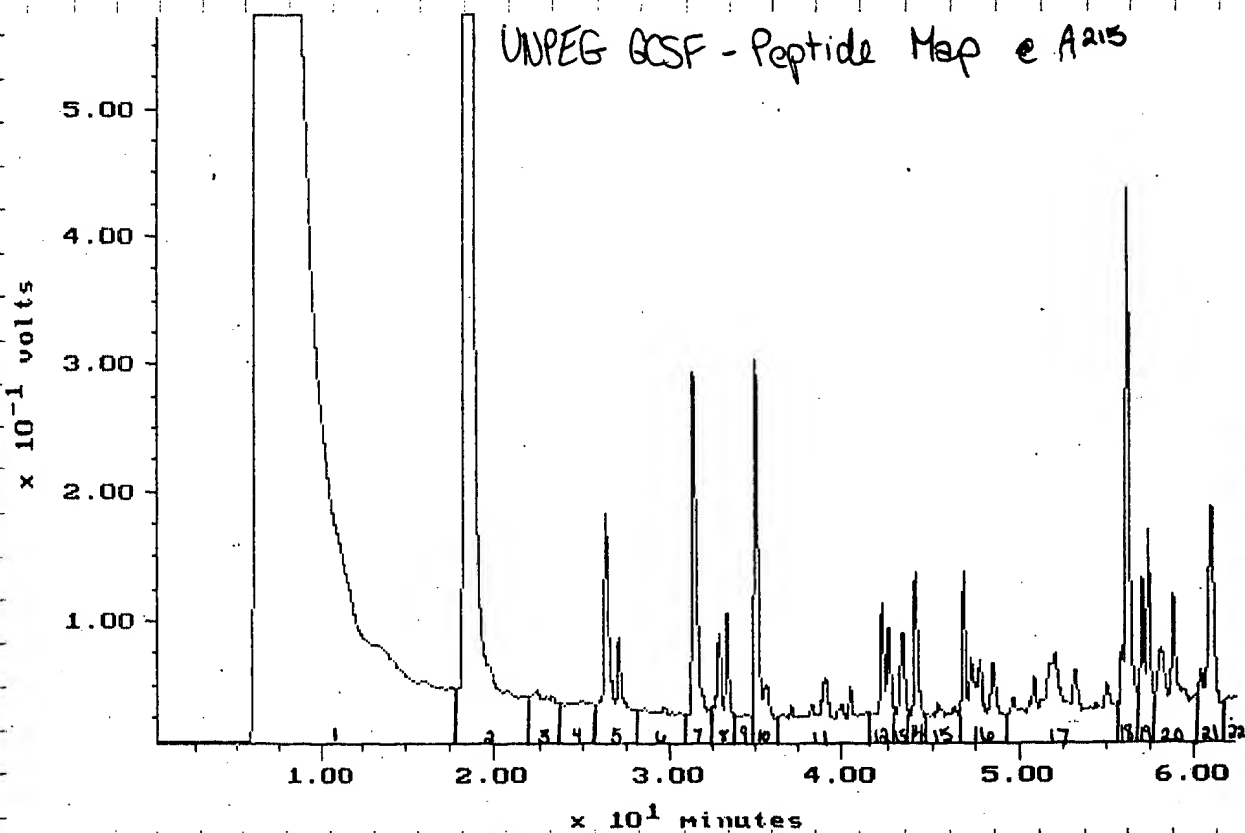
From Page No. 55 baseline monitored & sketched. Fractions were collected by hand & numbered:

UNPEG GCSF MAP

(sketched during run)



UNPEG GCSF - Peptide Map e A215



To Page No. 57

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Farnham

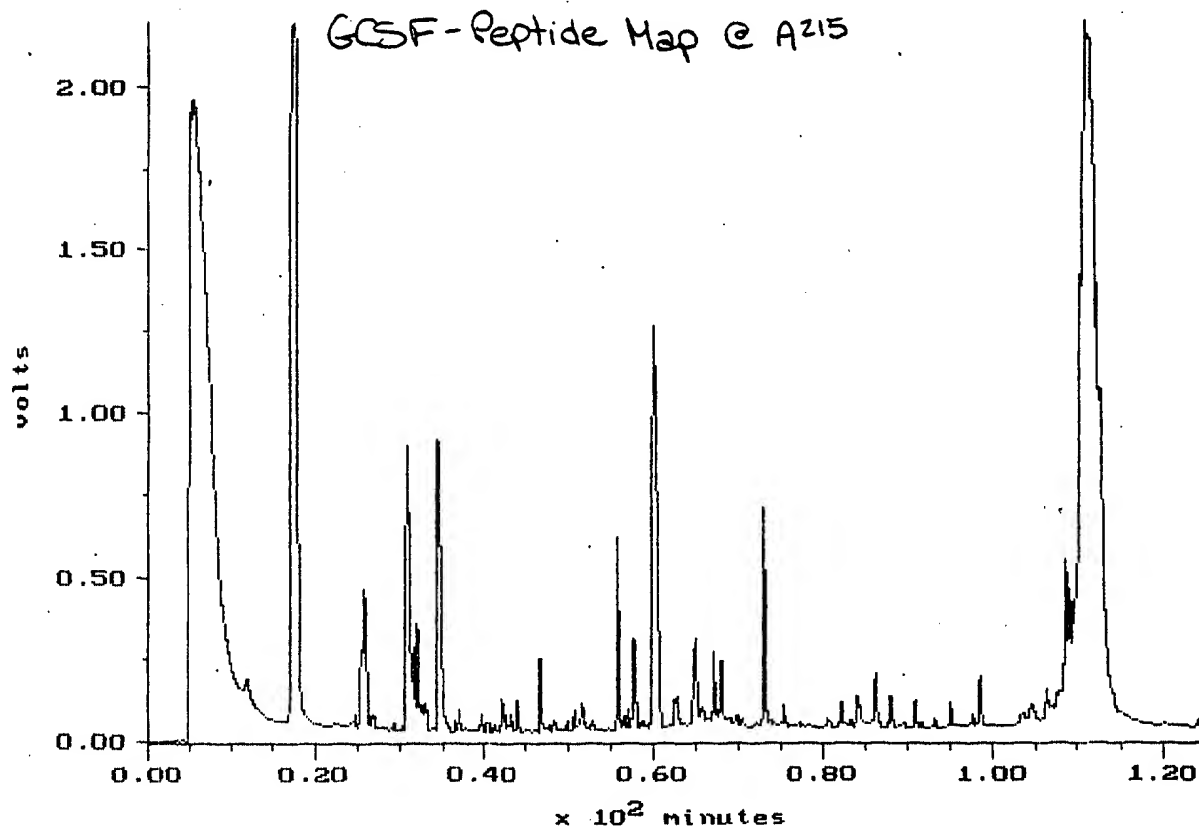
Recorded by

Date

TITLE _____

From Page No. 54

The GCSF standard was run for comparison. Nothing was collected.

To Page No. 58

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Date

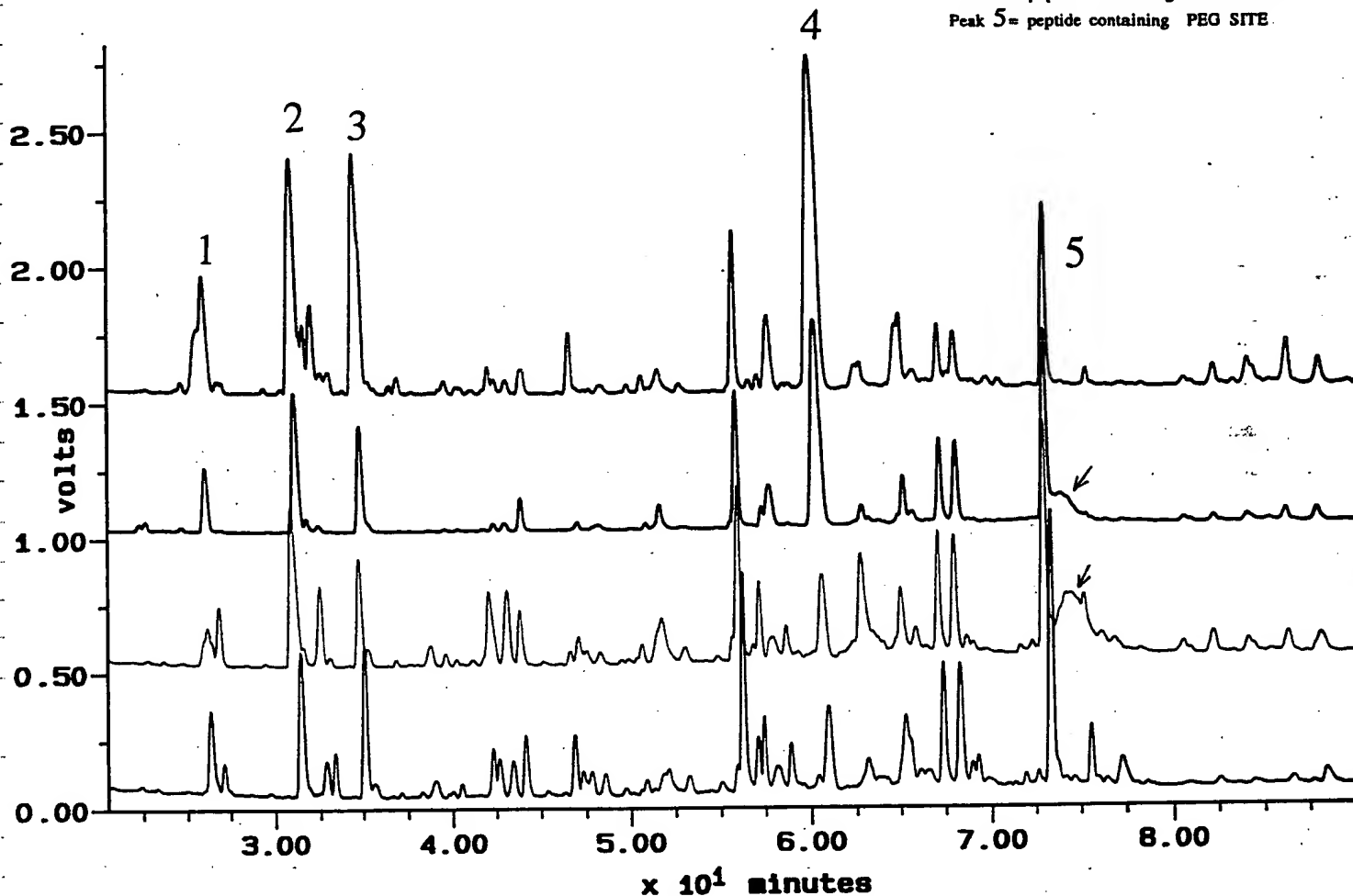
Recorded by

From Page No. 51

UV chromatograms of peptide maps of GCSF, mono pegylated lys35 GCSF lot #6951-24 (containing the unknown mono pegylated species), purified mono pegylated lys35 GCSF, and the unpegylated GCSF derived from the unknown mono pegylated species.

— GCSF
— LYS35 MIX
— LYS35 PURE
— UNPEG GCSF

Peak 1 = peptide containing LYS35
Peak 2 = peptide containing LYS24
Peak 3 = peptide containing LYS41
Peak 4 = peptide containing N-TERMINUS
Peak 5 = peptide containing PEG SITE

To Page No. 59

Witnessed & Understood by me,

Anne H. Gar

Date

Invented by

Christine Farmer

Date

Recorded by

111

TITLE _____

REDACTED

Project No. 102683

Book No. 6951

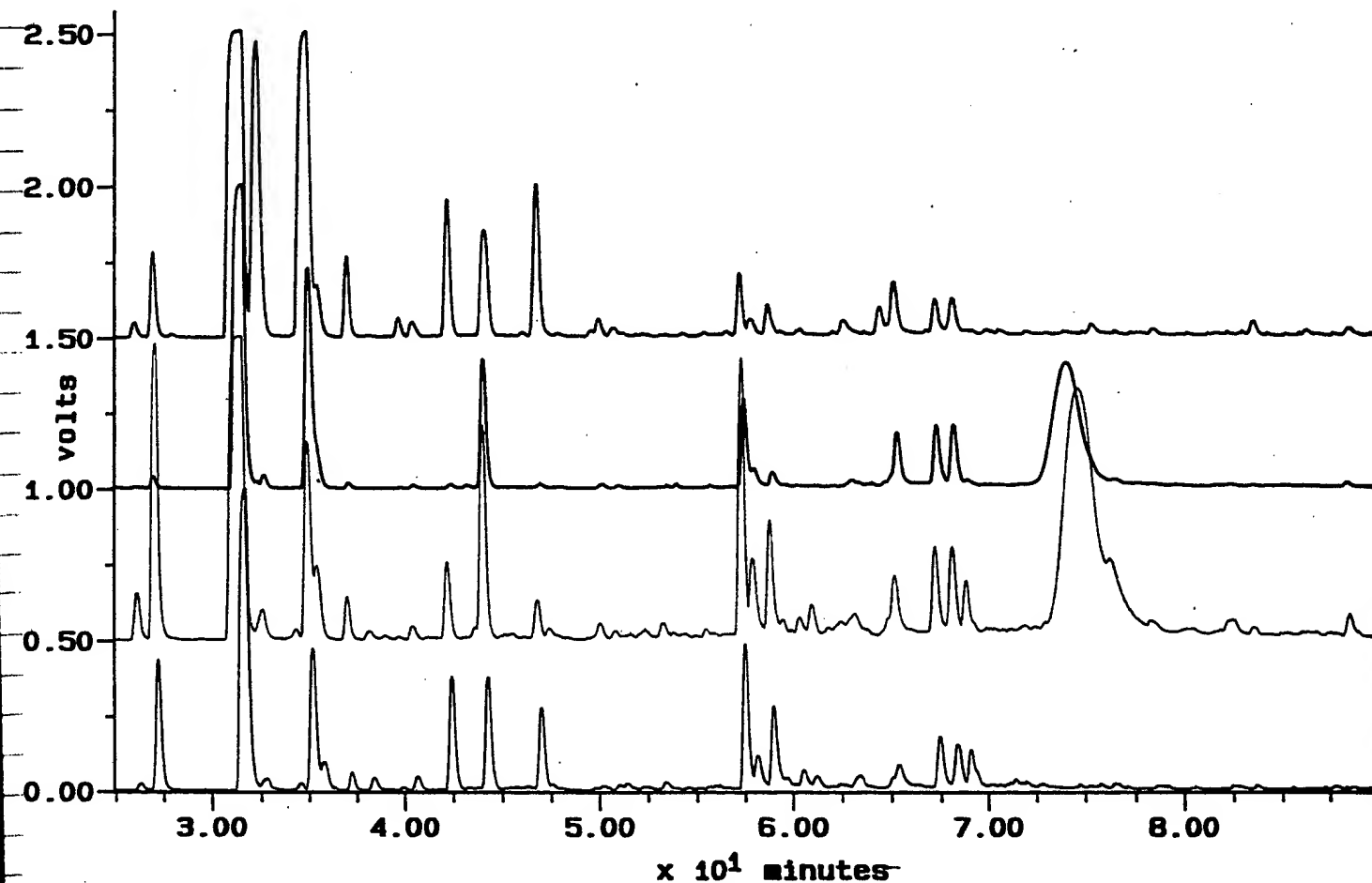
59

From Page No. 58

FLORESCENT chromatograms of peptide maps of GCSF, mono pegylated lys35 GCSF lot #6951-24 (containing the unknown mono pegylated species), purified mono pegylated lys35 GCSF, and the unpegylated GCSF derived from the unknown mono pegylated species.

Peak * = peptide with florescently labeled cys37

— GCSF
— LYS35 MIX
— LYS35 PURE
— UNPEG. GCSF



To Page No. 60

Witnessed & Understood by me,

Amel H. Gane

Date

Invented by

Christine Johnson

Recorded by

Date

From Page No. 59

REDACTED

Discussion:

The separation of the mono peg-lys35 from the unpegylated unknown site was successful as seen by the FPLC chromatogram (page 50). However, there appeared to be some aggregate which may or may not have been partially composed of the unknown species. Also, this overlay shows that the unpegylated unknown species is somewhat hydrodynamically larger in size than unmodified GCSF.

The size exclusion HPLC data confirms that the purification was successful aside from the small amount of aggregate collected with the purified lys35 peak (page 53). The SDS PAGE also supports successful separation (page 51). Although it looks like there is more of a mixture than before, after integration of the peaks, it shows that there is actually a 75% reduction in the unpegylated band, and the fact that the band is there at all may be attributed to the aggregate contained in the purified sample.

The fact that this is purified lys35 and not the unknown mono pegylated species was supported by peptide mapping. As seen in the UV detector overlay (page 58) the peak assigned to lys35 is diminished in both the mixture chromatogram and the purified chromatogram, and both also contain a PEG peak signifying the pegylation of lys35. The PEG peak (fxn 31 and 32, page 55) was collected for sequencing just for confirmation. The unpegylated unknown species chromatogram does not have a diminished lys35 peak and also contains no PEG peak. The reason for the diminished n-terminus peak in the lys35 purified and unpegylated species chromatograms is unknown.

The florescence detector overlay also supports that the purified pegylated species is lys35 (page 59). Before loading the samples onto the column they were treated with ABD-F which would florescently label cysteine37, which is on the same peptide as pegylated lys35. In the lys35 purified chromatogram the peg peak is labeled proving that it is indeed pegylated on the peptide containing lys35 and cysteine37. Another indication that the species was purified is that lys35's florescent peak is about twice the size of the mixture's florescent peak, which correlates with the fact that the mixture of mono pegs was about 50/50.

To Page No. 61

Witnessed & Understood by me,

Anne H. Allen

Date

Invented by

Christine Turner

Date

Recorded by

TITLE

From Page No. 60

REDACTED

SEQUENCING:

Fractions 31 & 32 were dried down in a speed vac. Stored at 4°C

This sample was submitted to Scott Lauren for AAA and N-Terminal Sequencing.

AMGEN

MEMORANDUM

DATE:

TO: Mike Treuheit

FROM: Scott L. Lauren and Michael F. Rohde

Report: 062993-SLL-40

RE: Amino Acid Analysis and N-Terminal Sequence of PEG-GCSF Lys-C Peptide

PEG-GCSF Lys-c peptide residues 21-40, our sample number 061093A, was submitted for amino acid analysis to determine if the sample is the correct G-CSF peptide of a lys-c digest and is lysine 34 modified. The sample was reconstituted in 100 uL 0.1%TFA. One aliquot of 80.0 uL was hydrolyzed according to Analytical Method A0130. The sample was reconstituted in 70.0 uL Norleucine dilution buffer, then run on a Beckman 6300 Amino Acid Analyzer. The determined amino acid composition compared to the theoretical composition, of the expected peptide from G-CSF, gave a correlation coefficient of 0.998.

A 20 uL aliquot was analyzed by N-terminal sequencing for 15 cycles. The expected peptide sequence was observed and the yield at lys 34 was less than one percent of the yield at adjacent residues.

The data supports that the sample is the G-CSF peptide, residues 21 through 40.

To Page No. 62

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Farnan

Date

Recorded by

REDACTED

From Page No. 6

MASS SPEC:

1 ml aliquots of the purified Lys35 PEG-GCSF and the UNPEG-GCSF species (from pages 48 + 49) were given to Bawergh Shaw in Protein Chemistry for time of flight mass spec. Also submitted was part of Ex 31 + 32 from page 55, the pegylated peptide of the purified Lys35 sample.

Kratos Kompact MALDI 3 V2.0.1 : Run E0434

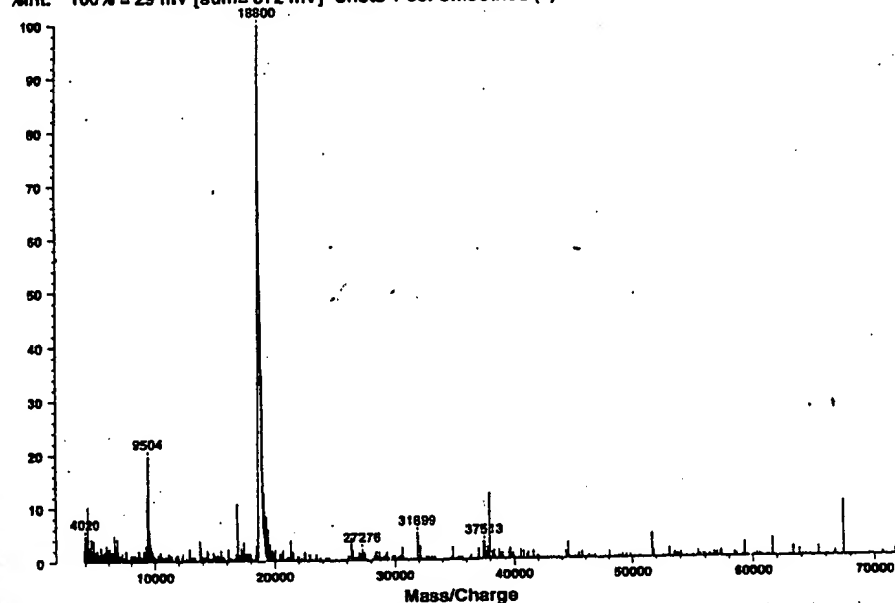
17:14 +Lin HI Pwr 44

T 6702 (0.1mg/ml)

Sample 7:

GCSF

%Int. 100% = 29 mV [sum= 872 mV] Shots 1-30: Smoothed (1)



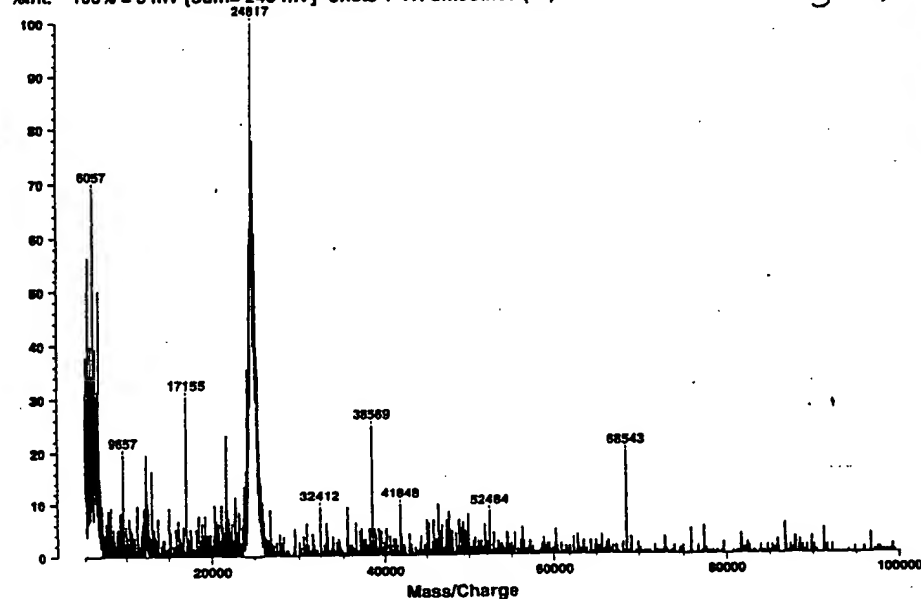
Kratos Kompact MALDI 3 V2.0.1 : Run E0415

17:27 +Lin HI Pwr 52

Sample 13:

Lys35
(1mg/ml)

%Int. 100% = 5 mV [sum= 243 mV] Shots 1-41: Smoothed (10)

To Page No. 63

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

TITLE _____

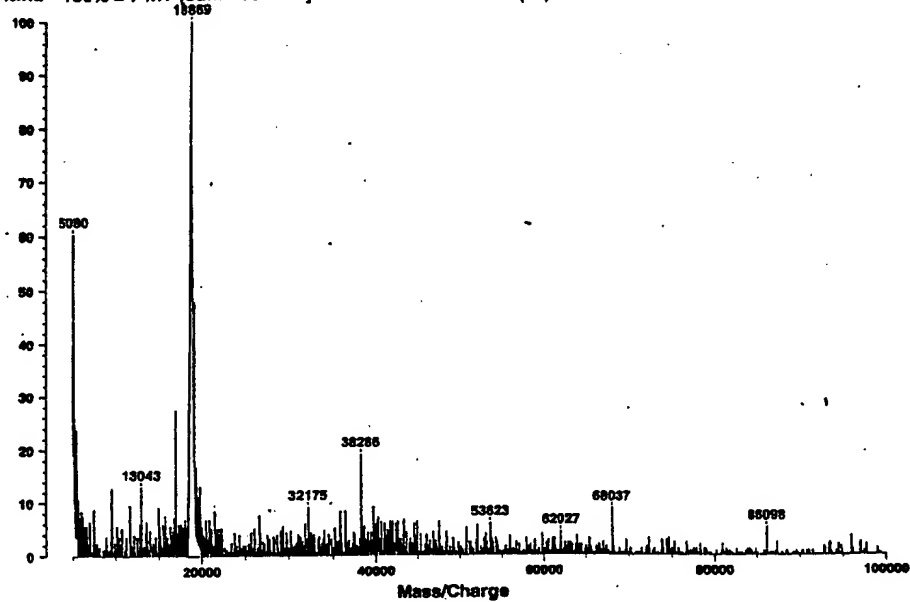
REDACTED

From Page No. 62Kratos Kompact MALDI 3 V2.0.1 : Run E0410
Sample 10:

17:10 +Lin HI Pwr 52

DNPEQ-GCxF
(0.1mg/ml)

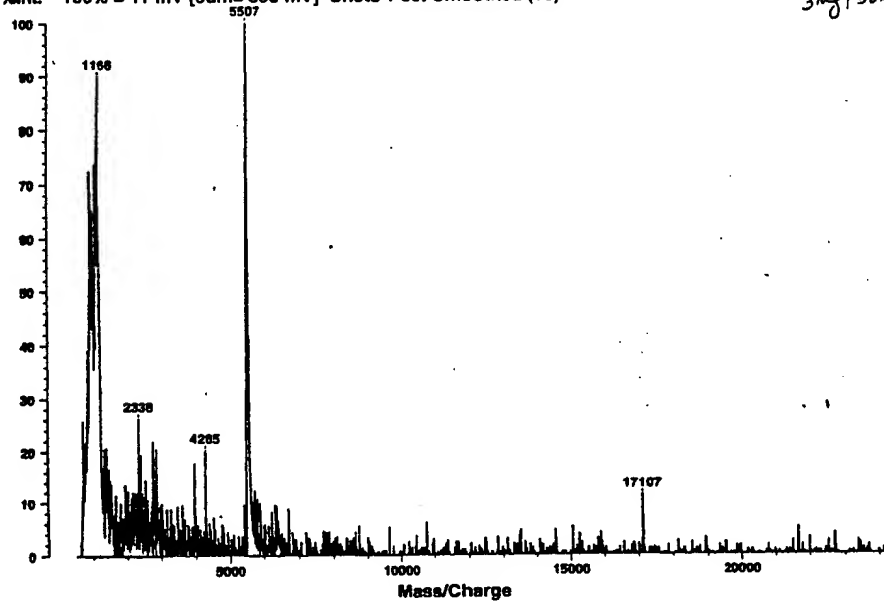
%Int. 100% = 7 mV (sum= 301 mV) Shots 1-39: Smoothed (10)

Kratos Kompact MALDI 3 V2.0.1 : Run E0428
Sample 7:

13:13 +Lin HI Pwr 47

peptide
Lys-35
peptide -
3mg/30ml

%Int. 100% = 11 mV (sum= 560 mV) Shots 1-50: Smoothed (10)

To Page No. 64

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 63 INVIVO BIOASSAY

The purified LYS35 PEG-GCSF and the UNPEG species from pages 48+49 were buffer exchanged into GCSF formulation buffer and an aliquot of each was diluted to 1 mL along w/ an aliquot of GCSF lot #T6702 and LYS35 PEG-GCSF lot #6951-24. The 1 mL samples + some of the buffer were sterile filtered and transferred into 5 mL sterile injection vials and sealed w/ 1.5 mm sterile rubber septa, all performed in a laminar flow hood. The samples were ready for in vivo assay.

Mono-Peg-GCSF Hamster Dosing Study No. G060293

Animals:

Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule:

single S.C. injection of 0.1 mL on the first day

Sacrifice Schedule:

Four animals from each group will be bled at 0.5, 1.0, 1.5, 2.0, 4.0, and 7.0 days following dosing. (Please note time if different than listed.)

Injection Notes:

Use one vial for each treatment group.
Fill a 1-mL syringe, and inject up to 10 hamsters.
No need to change syringe needles between hamsters within a single group.
Vials are overfilled by 1.1 mL to allow for losses during filling syringes.

Analysis:

- Complete Blood Count: to be done on the same day that the samples were collected. Samples exceeding the range of the blood cell counter (other than platelets) will be diluted and recounted.
- Blood smear slides: a thin, air-dried blood smear slide will be prepared and stained with a stain (Wright or equivalent) suitable for differential leukocyte analysis.

Group	Animal per Group	Injection mL	Aver. Wt. (Kg)	Vial Vol. (mL)	No. of Vials
1. Vehicle	24	0.1	0.1	3.5	1
2. GCSF T6702	24	0.1	0.1	3.5	1
3. 6951-24 lys35mix	24	0.1	0.1	3.5	1
4. LYS35 purified	24	0.1	0.1	3.5	1
5. UNPEG GCSF	24	0.1	0.1	3.5	1

To Page No. 65

Witnessed & Understood by me,

Anne H. Grace

Date

Invented by

Christina Turner

Date

Recorded by

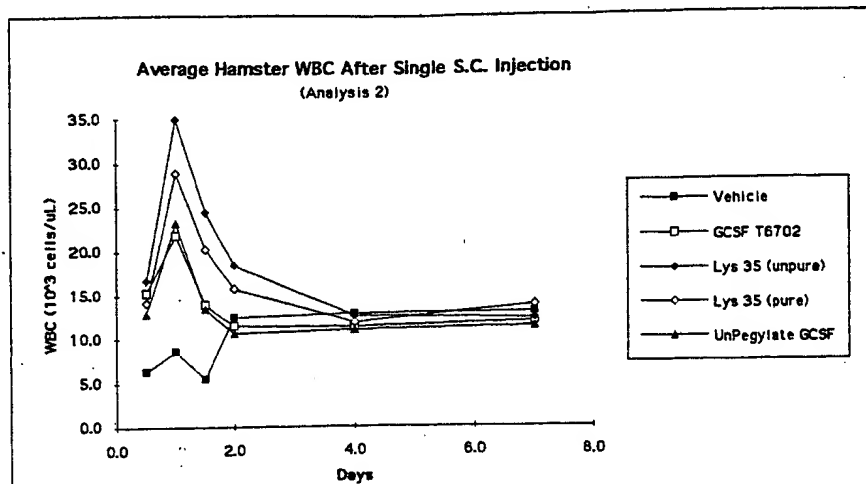
TITLE _____

From Page No. 14

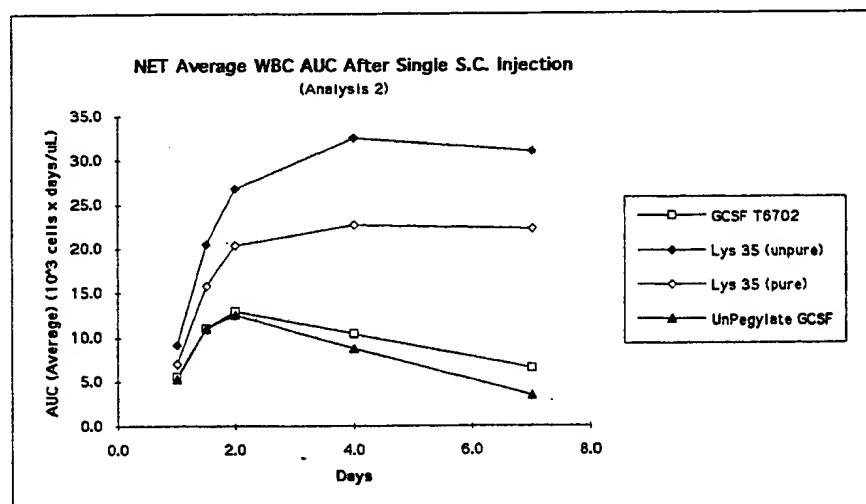
Table 1: Average WBC per Time Point (Days)

Group No.	Sample Code/Days	0.5	1.0	1.5	2.0	4.0	7.0	
1	Vehicle	6.4	8.7	5.6	12.6	12.9	13.0	#DIV/0!
2	GCSF T6702	15.4	22.0	14.1	11.7	11.4	12.0	#DIV/0!
3	Lys 35 (unpure)	16.8	35.0	24.6	16.7	12.6	12.3	#DIV/0!
4	Lys 35 (pure)	14.2	29.0	20.4	16.0	11.9	13.8	#DIV/0!
5	UnPegylate GCSF	13.0	23.4	13.6	10.8	11.0	11.3	#DIV/0!

REDACTED



Group No.	Sample Code/Days	1.0	1.5	2.0	4.0	7.0	
2	GCSF T6702	5.6	11.0	12.9	10.4	6.6	#DIV/0!
3	Lys 35 (unpure)	9.2	20.5	26.8	32.6	31.1	#DIV/0!
4	Lys 35 (pure)	7.0	15.8	20.4	22.7	22.2	#DIV/0!
5	UnPegylate GCSF	5.3	11.0	12.6	8.8	3.5	#DIV/0!

Discussion:

From the data it appears that the mixture (unstable site plus Lys35) is somewhat more active than the Lys35 by itself. These results are rather unexpected, yet they are conceivable when considering the locations of the receptor binding sites. (Receptor binding is believed to involve sites 20,24, and 26 on the A helix and site 47 on the little E helix.) If the unstable site is somehow less obstructive toward receptor binding than the Lys35, you might expect this species to have higher activity. (This theory assumes that the clearance rates and receptor turnover for all the mono peg-GCSF's are the same.)

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

From Page No. 2

REDACTED

Materials: MONO PEG-GSF @ LYS35 lot #6951-24

20mM NaOAc pH 4.0 (made w/ WFI) - A Buffer
20mM NaOAc, 1M NaCl pH 4.0 (made w/ WFI) - B Buffer

Pharmacia FPLC System
MONOS column #92312 from Pharmacia

BioSep SEC3000 column #50242
100mM NaPhos pH 6.9 (made w/ Milli Q)
Waters HPLC System

To Page No. 104

Witnessed & Understood by me,
Anne H. Gao

Date

Invented by
Christine Farnas

Date

Recorded by

11

From Page No. 66

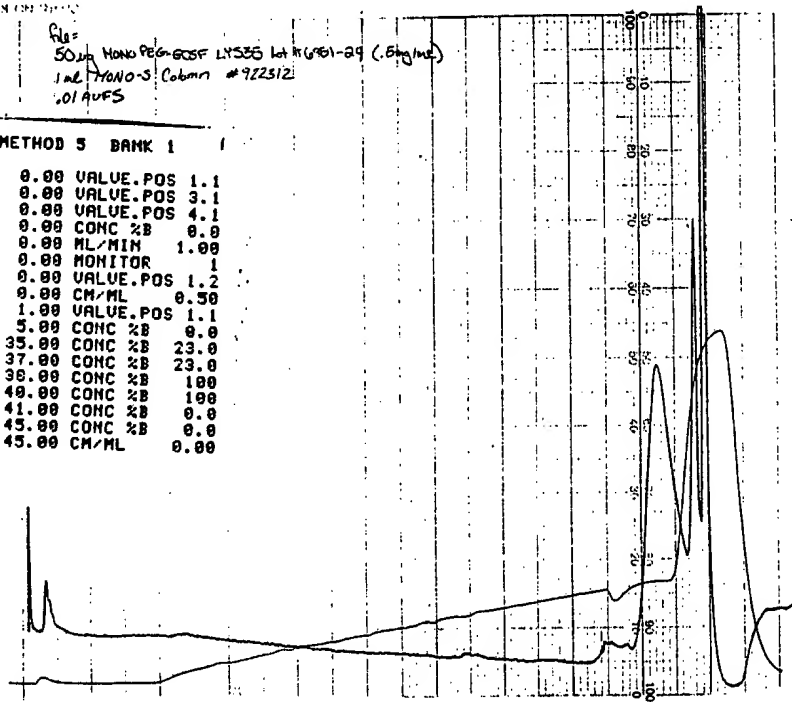
Procedure

50 µg of Mono PEG-GCSF 01535 lot #6951-24 was loaded onto a 100 µm loop. A linear gradient method was run to attempt to separate the different components of this lot of PEG-GCSF without degrading the one species by leaving the sample @ pH 4.0 during this assay. The process was repeated 7 times using a different gradient each time. The seventh time (File 061093-1) the various fractions of the peak were collected and run on SEC HPLC to see if any difference could be detected between the fractions. (see page 72). No significant conclusions were drawn from this data.

FILE 061093-1
RUN MONO PEG-GCSF 01535 LOT #6951-24 (6 µg/mL)
1 µL MONO-S Column #922312
0.01 AUFS

METHOD 5 BANK 1

0.00	VALUE POS	1.1
0.00	VALUE POS	3.1
0.00	VALUE POS	4.1
0.00	CONC %B	0.0
0.00	ML/MIN	1.00
0.00	MONITOR	1
0.00	VALUE POS	1.2
0.00	CM/ML	0.50
1.00	VALUE POS	1.1
5.00	CONC %B	0.0
35.00	CONC %B	23.0
37.00	CONC %B	23.0
38.00	CONC %B	100
40.00	CONC %B	100
41.00	CONC %B	0.0
43.00	CONC %B	0.0
45.00	CM/ML	0.00



To Page No. 68

Witnessed & Understood by me,

Anne H. Gao

Date

Invented by

Christine Ferran

Date

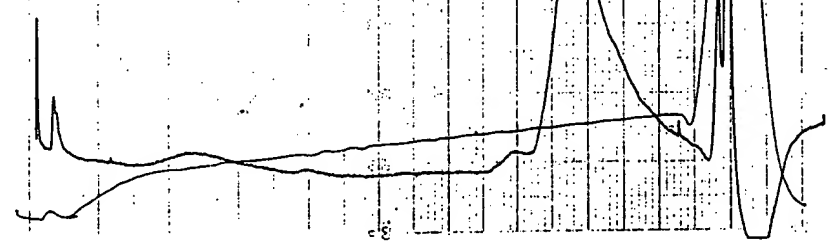
Recorded by

From Page No. 67

File: 50mg NMDP-6GSE LVS35 #1951-24 (5mg/ml)
1ml MONO-S column #922312
01 ABFS

METHOD 6 BANK 1 2

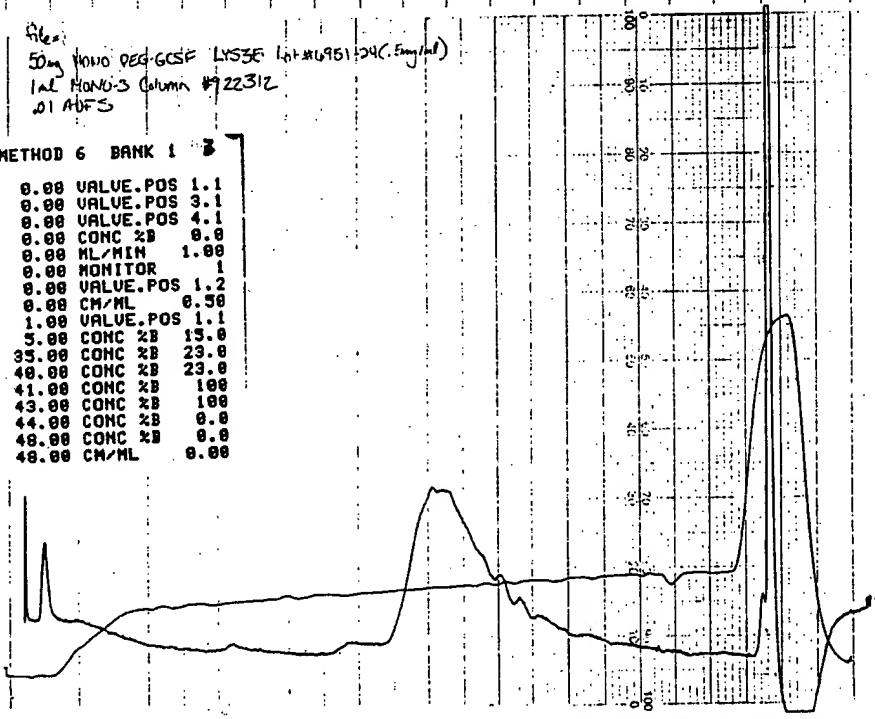
0.00 VALVE.POS 1.1
0.00 VALVE.POS 3.1
0.00 VALVE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 1.00
0.00 MONITOR 1
0.00 VALVE.POS 1.2
0.00 CM/ML 0.50
1.00 VALVE.POS 1.1
5.00 CONC %B 10.0
35.00 CONC %B 23.0
37.00 CONC %B 23.0
38.00 CONC %B 100
40.00 CONC %B 100
41.00 CONC %B 0.0
45.00 CONC %B 0.0
45.00 CM/ML 0.00



File: 50mg NMDP-6GSE LVS35 #1951-24 (5mg/ml)
1ml MONO-S column #922312
01 ABFS

METHOD 6 BANK 1 3

0.00 VALVE.POS 1.1
0.00 VALVE.POS 3.1
0.00 VALVE.POS 4.1
0.00 CONC %B 0.0
0.00 ML/MIN 1.00
0.00 MONITOR 1
0.00 VALVE.POS 1.2
0.00 CM/ML 0.50
1.00 VALVE.POS 1.1
5.00 CONC %B 15.0
35.00 CONC %B 23.0
40.00 CONC %B 23.0
41.00 CONC %B 100
43.00 CONC %B 100
44.00 CONC %B 0.0
48.00 CONC %B 0.0
48.00 CM/ML 0.00



To Page No. 69

Witnessed & Understood by me,

Anne H. Gar

Date

Invented by

Christine Furson

Date

Recorded by

-1

TITLE _____

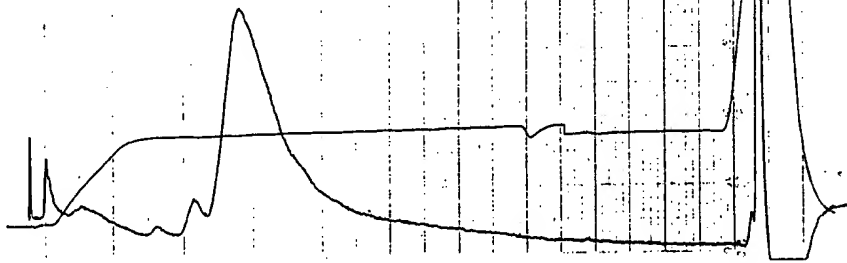
REDACTED

From Page No. 68

File #
50mg MONO PEG GCSF L5335 in #1851-24 (Signal)
1 mL MONO-S Column #922312
.01 PUPS

METHOD 6 BANK 1 4

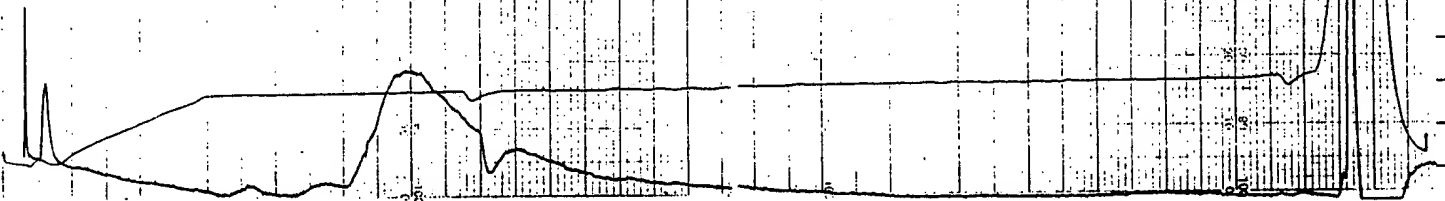
0.00	VALUE.POS	1.1
0.00	VALUE.POS	3.1
0.00	VALUE.POS	4.1
0.00	CONC %B	0.0
0.00	ML/MIN	1.00
0.00	MONITOR	1
0.00	VALUE.POS	1.2
0.00	CM/ML	0.50
1.00	VALUE.POS	1.1
5.00	CONC %B	28.0
35.00	CONC %B	23.0
40.00	CONC %B	23.0
41.00	CONC %B	100
43.00	CONC %B	100
44.00	CONC %B	0.0
48.00	CONC %B	0.0
48.00	CM/ML	0.00



File #
50mg MONO PEG GCSF L5335 in #1851-24 (Signal)
1 mL MONO-S Column #922312
.01 PUPS

METHOD 7 BANK 1 5

0.00	VALUE.POS	1.1
0.00	VALUE.POS	3.1
0.00	VALUE.POS	4.1
0.00	CONC %B	0.0
0.00	ML/MIN	1.00
0.00	MONITOR	1
0.00	VALUE.POS	1.2
0.00	CM/ML	0.50
1.00	VALUE.POS	1.1
10.00	CONC %B	18.0
10.00	ML/MIN	0.50
70.00	CONC %B	21.0
70.00	ML/MIN	1.00
75.00	CONC %B	23.0
76.00	CONC %B	100
78.00	CONC %B	100
79.00	CONC %B	100
83.00	CONC %B	0.0
83.00	CM/ML	0.00



To Page No. 70

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

Christine Jones

Date

Recorded by

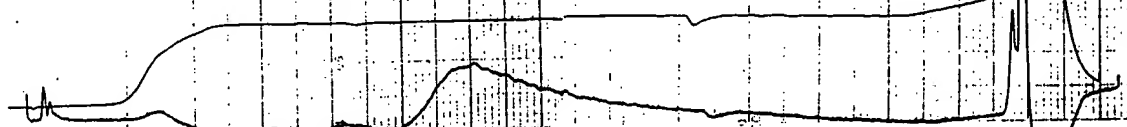
From Page No. 69

REDACTED

File: EDU HOLD PEG-GOSF-LYS-35 1P #451-24 (1mg/ml)
 1 ML HOLD S Column #92512
 01 PUS

METHOD 7 BANK 1

0.00 VALUE.POS 1.1
 0.00 VALUE.POS 3.1
 0.00 VALUE.POS 4.1
 0.00 CONC %B 0.0
 0.00 ML/MIN 0.50
 0.00 MONITOR 1
 0.00 VALUE.POS 1.2
 0.00 CM/ML 0.50
 1.00 VALUE.POS 1.1
 10.00 CONC %B 10.0
 50.00 CONC %B 10.0
 55.00 CONC %B 23.0
 56.00 CONC %B 100
 58.00 CONC %B 100
 59.00 CONC %B 0.0
 62.00 CONC %B 0.0
 63.00 CM/ML 0.00

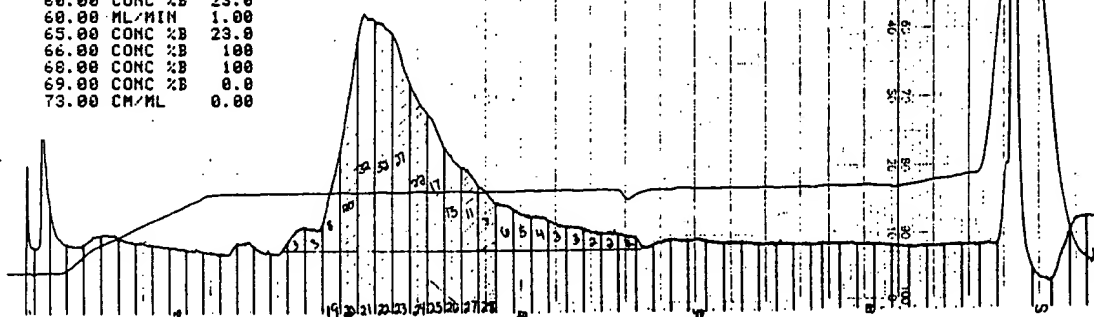


File: EDU HOLD PEG-GOSF-LYS-35 1P #451-24 (1mg/ml)
 1 ML HOLD S Column #92512
 01 PUS
 Fraction size: 1 ml

METHOD 6 BANK 2

0.00 VALUE.POS 1.1
 0.00 VALUE.POS 3.1
 0.00 VALUE.POS 4.1
 0.00 CONC %B 0.0
 0.00 ML/MIN 1.00
 0.00 MONITOR 1
 0.00 VALUE.POS 1.2
 0.00 CM/ML 0.50
 1.00 VALUE.POS 1.1
 10.00 CONC %B 10.0
 10.00 ML/MIN 0.50
 60.00 CONC %B 23.0
 60.00 ML/MIN 1.00
 65.00 CONC %B 23.0
 66.00 CONC %B 100
 68.00 CONC %B 100
 69.00 CONC %B 0.0
 73.00 CM/ML 0.00

(20x5) (xmg/ml) = (0.01) (0.01)

To Page No. 71

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Ann H. Gae

Christine Turner

TITLE _____

From Page No. 70

REDACTED

HPLC SEC:

Request # 1246

Date Submitted: _____

Analytical Results Needed by: _____

Submitted by: Chris Farrar

Protein (Analyte): PEG-GCSF PLC 20 01093-1

Analysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition: _____

Potential Interferences (polymers, detergents, UV-absorbing species, etc.): _____

Operator: CF

Column: SEC3000 #50242

Method: 3004

Date Results Reported: _____

Instrument: # 2

No.	Inj. vol.	File Name	Conc mg/ml	Sample Identification	No.	Inj. Vol	File Name	Conc mg/ml	Sample Identification
1	5	53 3113	—	STD	25				
2	50	3114	.1	GCSF 17.702	26				
3	100	3115	.1	"	27				
4	200	3116	.1	"	28				
5	100	3117	.015	FXN 17+18	29				
6		3118	.015	" 19+20	30				
7		3119	.150	" 21+22	31				
8		3120	.110	" 23+24	32				
9		3121	.070	" 25+26	33				
10		3122	.040	" 27+28	34				
11		3123	.015	" 29+30	35				
12		3124	.015	" 31+32	36				
13	✓	3125	.115	" 33+34	37				
14	100	3126	.1	GCSF 17.702	38				
15	5	3127	—	STD	39				
16					40				
17					41				
18					42				
19					43				
20					44				
21					45				
22					46				
23					47				
24					48				

Notes: _____

To Page No. 72

Witnessed & Understood by me,

Date

Invented by

Date

Christine Farrar
Recorded by

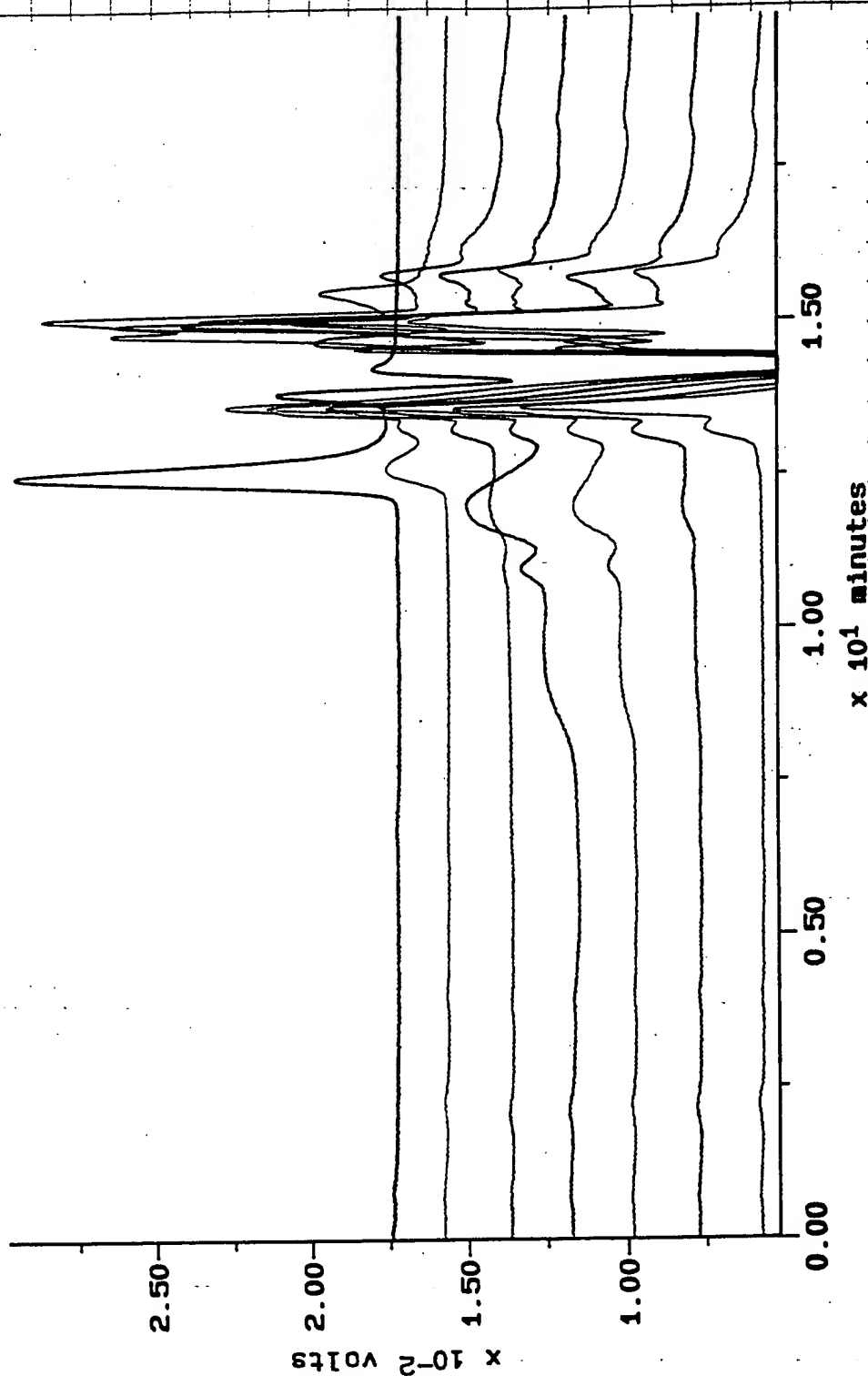
Anne H. Garcia

From Page No. 11

HPLC SEC Chromatograms @ λ_{280} of Peak FXN's from FPLC run 06/09/93-1

— FXN 23, 24
— FXN 25, 26
— FXN 27, 28

— GCSF
— FXN 58, 59
— FXN 19, 20
— FXN 21, 22



To Page No. X

Witnessed & Understood by me,

Anne H. Garra

Date _____

Invented by

Christine Farnas

Recorded by _____

Date _____

From Page No. X

REDACTED

Materials: GCSF lot TL6702
 MONO-PEG-GCSF @ N-TERM lot # 6951-23
 MONO-PEG-GCSF @ LYS41 lot # 6951-25
 MONO-PEG-GCSF @ LYS35 from pages 47-65
 10mM NaOAc, 5% Mannitol, .004% Tween 80 pH 4.0 (made w/ WFI)
 - GCSF formulation buffer
 120 Male Golden Syrian Hamsters, 90-100g
 1 ml syringes (x15)
 25 G5/8 needles
 5 ml syringes (x120)
 22 G1 needles (x120)
 CO₂ tank + CO₂
 Sysmex Blood Cell Counter
 Slides (x120) blood smear

To Page No. 74

Witnessed & Understood by me,

Date

Invented by

Date

Christine Tarran
 Recorded by

Anne H. Gar

REDACTED

From Page No. 73

Procedure:

The purified LYS35 PEG-GCSF from pages 47-65 was buffer exchanged into GCSF formulation buffer and an aliquot of this solution was diluted to 1mg/ml along w/ an aliquot of GCSF lot #176202, MONO PEG-GCSF lot #16951-23, and MONO PEG-GCSF lot #16951-25. The 1mg/ml samples + some of the buffer were sterile filtered and transferred into 5ml sterile injection vials and sealed w/ 13mm sterile rubber seals, all performed in a laminar flow hood. The samples were taken to vivarium for inj.

Mono-Peg-GCSF Hamster Dosing Study No. G061493

Animals:

Male Golden Syrian Hamsters, 90-100 g

Dosing Schedule:

single S.C. injection of 0.1 mL on the first day

Sacrifice Schedule:

Four animals from each group will be bled at 0.5, 1.0, 1.5, 2.0, 4.0, and 7.0 days following dosing. (Please note time if different than listed.)

Injection Notes:

Use one vial for each treatment group.
Fill a 1-mL syringe, and inject up to 10 hamsters.
No need to change syringe needles between hamsters within a single group.
Vials are overfilled by 1.1 mL to allow for losses during filling syringes.

Analysis:

- Complete Blood Count: to be done on the same day that the samples were collected. Samples exceeding the range of the blood cell counter (other than platelets) will be diluted and recounted.
- Blood smear slides: a thin, air-dried blood smear slide will be prepared and stained with a stain (Wright or equivalent) suitable for differential leukocyte analysis.

Group	Animal per Group	Injection mL	Aver. Wt. (Kg)	Vial Vol. (mL)	No. of Vials
1. Vehicle	24	0.1	0.1	3.5	1
2. GCSF T6702	24	0.1	0.1	3.5	1
3. N-TERM 6951-23	24	0.1	0.1	3.5	1
4. LYS35 purified	24	0.1	0.1	3.5	1
5. LYS41 6951-25	24	0.1	0.1	3.5	1

To Page No. 75

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Anne H. Garcia

Date

Invented by

Christine Farnan

Date

Recorded by

TITLE

From Page No. 74

White Blood Cell Data:

REDACTED

Filename: Mono-PEG-GCSF Hamster Median WBC for Study #G001493

Note: Calculations Based on AVERAGING Points

Parameter:

WBC

Species:

Golden Syrian Hamster, Male

Test Material:

GCSF LOT #T6702, PEG-G-CSF LOT#6951-23, #6951-25, purified LYS35 pegylated GCSF

Study Dates:

10 mM NaOAc, 5 % mannitol, 0.004 % Tween 80, pH 4.0

Vehicle:

Group	Dose (ug/kg)	Animal No.	0.5	1.0	1.5	2.0	4.0	7.0
I. Vehicle		1	7.9		9.3	13.6	16.4	8.7
		2	16.7	13.6	8.0	7.0	9.5	7.6
		3	3.8	13.0	6.4	10.7	11.2	10.3
		4	6.3	13.5	4.6	12.2	11.0	11.5
		5						
		Min =	3.8	13.0	4.6	7.0	9.5	7.6
		Max =	16.7	13.6	9.3	13.6	16.4	11.5
		Average =	8.7	13.4	7.1	10.9	12.0	9.5
		n =	4.0	3.0	4.0	4.0	4.0	4.0
		Std. Dev. =	5.6	0.3	2.0	2.8	3.0	1.7
		SEM =	2.8	0.2	1.0	1.4	1.5	0.9
		Average AUC =		5.5	10.6	15.1	38.0	70.3
II. GCSF T6702	100	1	15.0	24.3	12.9	12.2	6.6	13.7
		2	23.0	39.7	7.2	10.5	11.3	11.9
		3	23.5	29.0	12.7	16.6	11.4	13.4
		4	18.5	29.7	11.4	10.8	8.9	13.0
		5						
		Min =	15.0	24.3	7.2	10.5	6.6	11.9
		Max =	23.5	39.7	12.9	16.6	11.4	13.7
		Average =	20.0	30.7	11.1	12.5	9.8	13.0
		n =	4.0	4.0	4.0	4.0	4.0	4.0
		Std. Dev. =	4.0	6.5	2.7	2.8	2.3	0.8
		SEM =	2.0	3.2	1.3	1.4	1.1	0.4
		Average AUC =		12.7	23.1	29.0	51.1	84.9
		Net Average AUC =	0.0	7.2	12.5	13.9	13.1	14.6
III. N-TERM	100	1			26.0	23.5	9.6	15.2
		2	11.5	20.6	21.8	23.3	8.3	
		3	21.4	22.3	27.2	23.9		15.4
		4	24.0	36.3		24.0	14.4	13.6
		5						
		Min =	11.5	20.6	21.8	23.3	8.3	13.6
		Max =	24.0	36.3	27.2	24.0	14.4	15.4
		Average =	19.0	26.4	25.0	23.7	10.8	14.7
		n =	3.0	3.0	3.0	4.0	3.0	3.0
		Std. Dev. =	6.6	8.6	2.8	0.3	3.2	1.0
		SEM =	3.8	5.0	1.6	0.2	1.9	0.6
		Average AUC =		11.3	24.2	36.4	70.8	109.1
		Net Average AUC =		5.8	13.6	21.3	32.8	38.7
IV. LYS 35 (pure)	100	1	15.7	33.1	15.3	13.7		11.8
		2	17.9	33.2	24.1	10.1	9.6	10.5
		3	17.3	31.3	23.6	13.2	11.6	11.3
		4		36.1	23.5	18.8	11.3	8.8
		5		34.5		16.6		
		Min =	15.7	31.3	15.3	10.1	9.6	8.8
		Max =	17.9	36.1	24.1	18.8	11.6	11.8
		Average =	17.0	33.6	21.6	14.5	10.8	10.6
		n =	3.0	5.0	4.0	5.0	3.0	4.0
		Std. Dev. =	1.1	1.8	4.2	3.3	1.1	1.3
		SEM =	0.7	0.8	2.1	1.5	0.6	0.7
		Average AUC =		12.7	26.5	35.5	60.8	93.0
		Net Average AUC =		7.1	15.6	20.4	22.8	22.6
V. LYS 41	100	1	20.5	25.5	7.8	11.2		10.5
		2		23.9	4.4	11.2	10.9	10.4
		3	15.2	24.7	11.6	12.1	7.1	10.4
		4	18.6	22.8	11.3	11.7	10.4	10.9
		5		25.3				
		Min =	15.2	22.8	4.4	11.2	7.1	10.4
		Max =	20.5	25.5	11.6	12.1	10.9	10.9
		Average =	18.1	24.4	8.7	11.8	9.5	10.6
		n =	3.0	5.0	4.0	4.0	3.0	4.0
		Std. Dev. =	2.7	1.1	3.4	0.4	2.1	0.2
		SEM =	1.6	0.5	1.7	0.2	1.2	0.1
		Average AUC =		10.6	18.9	24.0	45.0	75.0
		Net Average AUC =		5.1	8.3	8.9	7.0	4.7

To Page No. 76

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Date

Invented by

Christina Janssen

Date

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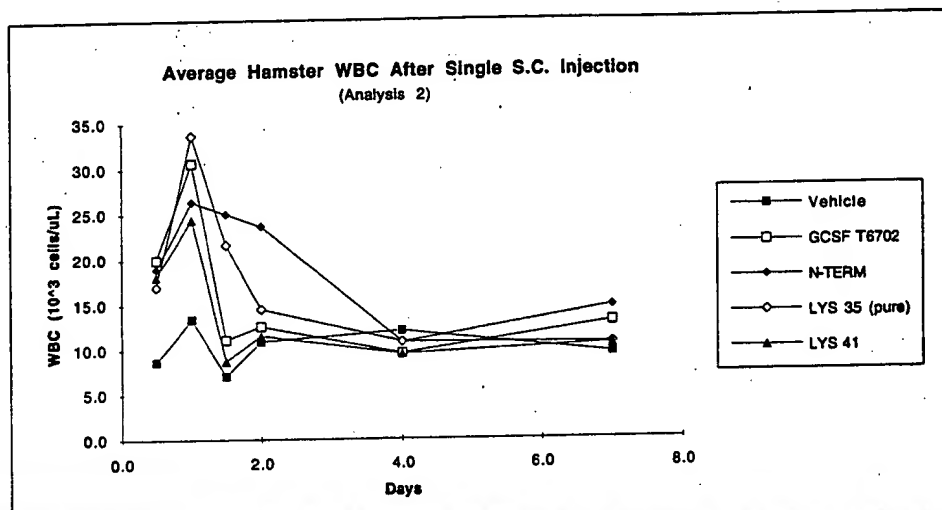
From Page No. 75

Average White Blood Cell Count per Time point:

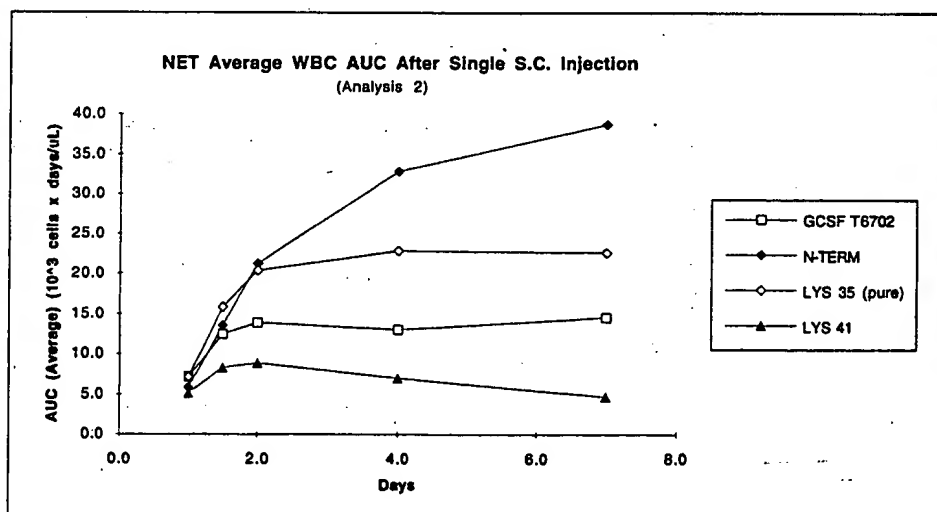
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Table1: Average WBC per Time Point (Days)

Group No.	Sample Code/Days	0.5	1.0	1.5	2.0	4.0	7.0
1	Vehicle	8.7	13.4	7.1	10.9	12.0	9.5
2	GCSF T6702	20.0	30.7	11.1	12.5	9.8	13.0
3	N-TERM	19.0	26.4	25.0	23.7	10.8	14.7
4	LYS 35 (pure)	17.0	33.6	21.6	14.5	10.8	10.6
5	LYS 41	18.1	24.4	8.7	11.6	9.5	10.6



Group No.	Sample Code/Days	1.0	1.5	2.0	4.0	7.0
2	GCSF T6702	7.2	12.5	13.9	13.1	14.8
3	N-TERM	5.8	13.6	21.3	32.8	38.7
4	LYS 35 (pure)	7.1	15.8	20.4	22.8	22.6
5	LYS 41	5.1	8.3	8.9	7.0	4.7

To Page No. 77

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From Page No. 10Analysis:

A White Blood Cell Count was taken of the collected blood samples from each hamster at each time point. Also, serum samples from each group at each time point were pooled and placed at -80C for further analysis by SDS PAGE at some future time.

Discussion:

The data confirms that the N-terminally pegylated form of mono peg-GCSF is the most active. It also confirms the data from the previous animal study which suggests that the mixture of the Lys35 form and the unstable site form is more active than the Lys35 form by itself (page 65). In vivo data for the Lys35 mixture form compared to the N-term and Lys41 forms of mono pegylated GCSF was obtained in February (page 19).

To Page No. X

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Anne H. Gao

Date

Invented by

Christine Farrer

Date

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From Page No. X

REDACTED

Materials: GCSF lot # T6702 2.91mg/ml
SCM-MPEG UCC lot #84-M from Union Carbide
N-Term PEG-GCSF lot #7559-01

Amicon stirred cell 50ml
43mm YM10 membrane/Amicon
WFI pH 4.0

Waters HPLC system
BioSep SEC3000 #52491 / Phenomenex
Pharmacia FPLC system
MONO S column #9304079

20mM NaOAc pH 4.0 - A buffer for MONO S column runs
20mM NaOAc, 1M NaCl pH 4.0 - B buffer for MONO S column runs
100mM NaPhos pH 6.9, buffer for SEC HPLC run

500mM Bicine pH 8.0 (made w/ WFI)
500mM NaPhos pH 6.0 (made w/ WFI)
500mM NaPhos pH 5.75 (made w/ WFI)

To Page No. 79

Witnessed & Understood by me,

Date

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Date

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Anne H. Gao

Christine Furrer

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From Page No. 78

Procedure:

~20ml of GCSF lot T16702 (2.91mg/ml) were concentrated to ~10ml in a 50ml Amicon stirred cell using a PM10 43mm membrane. The GCSF was then buffer exchanged into WFI pH 4.0 via a pressurized reservoir connected to the stirred cell. 6 reagent volumes (~60mls) were exchanged. The GCSF was then concentrated down to 12.5mg/ml (~4.5ml). 500ul of Bicine pH 8.0 buffer was added to 2ml of the 12.5mg/ml GCSF to give 2.5ml of GCSF 10mg/ml pH 8.0. 500ul of Naphos pH 6.0 buffer was added to another 2ml of the 12.5mg/ml GCSF to give 2.5ml of 10mg/ml GCSF pH 6.0. Then a specific amount of PEG was weighed into 4 4ml vials and the amount of GCSF needed to give a certain reaction ratio was calculated using the following equation:

$$\left(\frac{\text{X mg PEG}}{\text{Log mmol PEG}} \right) \div (\text{RXN ratio}) \times (18.8 \mu\text{mol GCSF}) = \text{mg GCSF to add}$$

	RXN ratio	PEG mg	GCSF mg
1) pH 6.0	3	4.1	4.28
2) pH 6.0	6	7.9	4.13
3) pH 8.0	15	2.3	4.80
4) pH 8.0	3	4.2	4.39

The appropriate amount of GCSF was added to PEG and the reactions were stirred at room temperature for 1 hour. They were then diluted 5X w/ WFI and their pH was adjusted to ~4. 15ul of each reaction mixture was then run on SEC HPLC to determine the what percentage of the solutions was pegylated and what percentage of the pegylated GCSF was mono pegylated. The percentages for reaction at pH 6.5.75 with a 15X reaction ratio were taken from the chromatograms for N-Term PEG-GCSF lot #7559-01. (PAGE 80+81)

5mg of each reaction mixture was run on FPLC mono-S column to determine what percentage of the mono pegylated species was N-Term. Again, the percentage for reaction at pH 5.75 with 15X reaction ratio were taken from the chromatograms for N-Term PEG-GCSF lot #7559-01. (PAGE 82)

To Page No. 80

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Date

Invented by

Date

Recorded by

Anne H. G.

Christine Johnson

om Page No. 71

HPLC SEC: Request Sheet + Spreadsheet for integration of peaks

Peak @ ~10.3 min = Di PEG-GCSF

Peak @ ~11.1 min = Mono PEG-GCSF

Peak @ ~12.5 min = Unmodified GCSF

REQUEST # 1268

REQUEST: 1268

FROM: CHRIS FARRAR

SUBMITTED:
REPORTED:

ANALYTE: MPEG-GCSF

METHOD: 3001

COLUMN: SEC3000 #52491

Date Submitted:

Analytical Results Needed by:

Submitted by: Chris Farrar

Protein (Analyte): PEG-GCSF

Analysis Requested (RP, SEC, IEX, etc.): SEC

Sample Buffer Composition:

Potential Interferences (polymers, detergents, UV-absorbing species, etc.):

Operator: CF

Column: SEC3000 #52491

Method: 3001

Date Results Reported:

Instrument # 2

No.	Inj. Vol.	File Name	Conc. mg/ml	Sample Identification	No.	Inj. Vol.	File Name	Conc. mg/ml	Sample Identification
1	50	SS-371	1	15X pH 5.7 RYN	25	30	SS-371	1	15X pH 5.7 RYN
2	5	375	1	15X pH 5.7 RYN	26	5	375	1	15X pH 5.7 RYN
3	15	376	2	15X pH 5.7 RYN	27	15	376	2	15X pH 5.7 RYN
4	1	377	1	15X pH 5.7 RYN	28	30	377	1	15X pH 5.7 RYN
5	1	378	1	15X pH 5.7 RYN	29	30	378	1	15X pH 5.7 RYN
6	1	379	1	15X pH 5.7 RYN	30	30	379	1	15X pH 5.7 RYN
7	1	380	1	15X pH 5.7 RYN	31	30	380	1	15X pH 5.7 RYN
8	1	381	1	15X pH 5.7 RYN	32	30	381	1	15X pH 5.7 RYN
9	1	382	1	15X pH 5.7 RYN	33	30	382	1	15X pH 5.7 RYN
10	1	383	1	15X pH 5.7 RYN	34	15	SS-371	2	15X pH 5.7 RYN
11	5	384	1	15X pH 5.7 RYN	35	1	384	1	15X pH 5.7 RYN
12	15	385	1	15X pH 5.7 RYN	36	1	385	1	15X pH 5.7 RYN
13	15	386	1	15X pH 5.7 RYN	37	1	386	1	15X pH 5.7 RYN
14	15	387	2	15X pH 5.7 RYN	38	1	387	1	15X pH 5.7 RYN
15	15	388	2	15X pH 5.7 RYN	39	1	388	1	15X pH 5.7 RYN
16	15	389	2	15X pH 5.7 RYN	40	1	389	1	15X pH 5.7 RYN
17	15	390	2	15X pH 5.7 RYN	41	1	390	1	15X pH 5.7 RYN
18	15	391	2	15X pH 5.7 RYN	42	1	391	1	15X pH 5.7 RYN
19	15	392	2	15X pH 5.7 RYN	43	1	392	1	15X pH 5.7 RYN
20	15	393	2	15X pH 5.7 RYN	44	1	393	1	15X pH 5.7 RYN
21	15	394	2	15X pH 5.7 RYN	45	1	394	1	15X pH 5.7 RYN
22	15	395	2	15X pH 5.7 RYN	46	1	395	1	15X pH 5.7 RYN
23	15	396	2	15X pH 5.7 RYN	47	1	396	1	15X pH 5.7 RYN
24	15	397	2	15X pH 5.7 RYN	48	1	397	1	15X pH 5.7 RYN

FILE	SAMPLE	TIME	AREA	PERCENT
83_3220	15X pH 5.75	10.308	868315	20.34
		11.142	2196403	51.45
		12.525	1204211	28.21
			4268929	
83_3190	3.0X pH 6 1hrCRT	10.317	83337	4.86
		11.142	535173	31.23
		12.517	1094917	63.90
			1713427	
83_3191	6.0X pH 6 1hrCRT	10.292	277192	14.80
		11.133	853469	45.55
		12.517	742832	39.65
			1873493	
83_3192	1.5X pH 8 1hrCRT	10.308	124863	7.11
		11.142	579183	32.97
		12.517	1052655	59.92
			1756702	
83_3193	3.0X pH 8 1hrCRT	10.300	439691	24.43
		11.133	787736	43.77
		12.508	572136	31.79
			1799563	

To Page No. 81

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Date

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Date

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TITLE _____

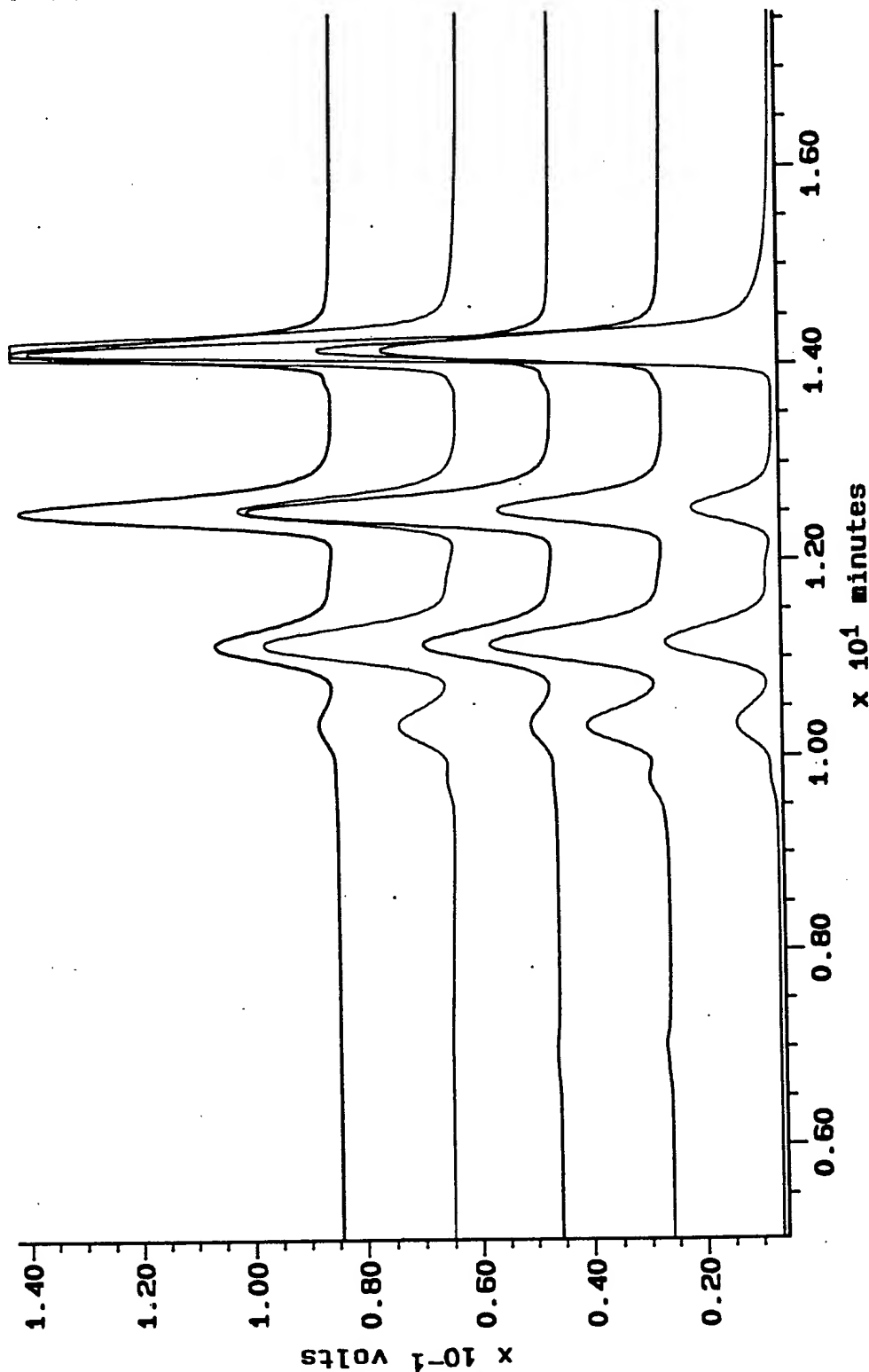
From Page No. 80

HPLC SEC Overlay:

Overlay of Chromatograms @ A280

— pH 6.0 3.0X
 — pH 6.0 6.0X
 — pH 8.0 1.5X
 — pH 8.0 3.0X

— pH 5.75 15X



To Page No. 82

Witnessed & Understood by me,

Anne H. Gae

Date

Invented by

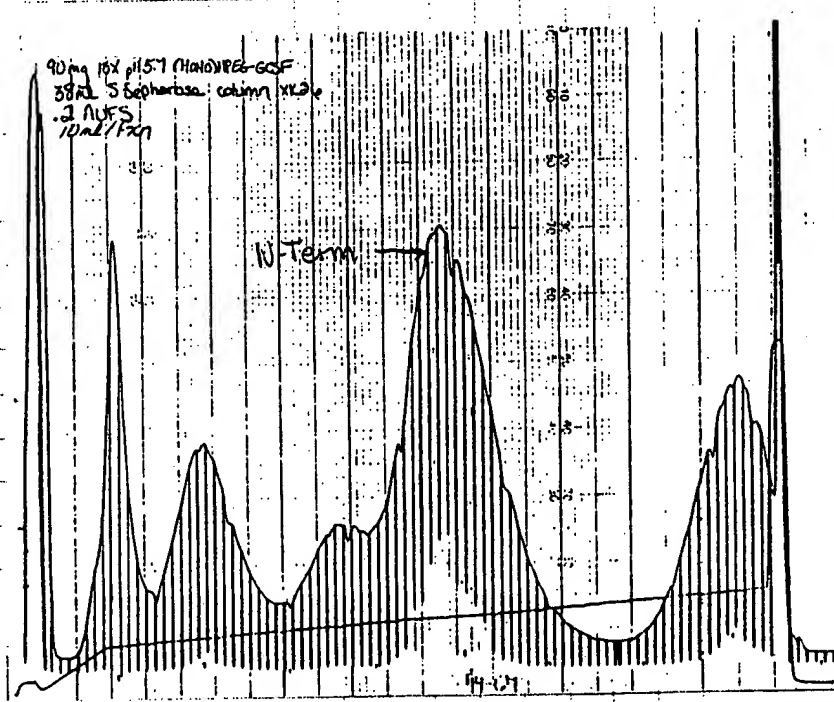
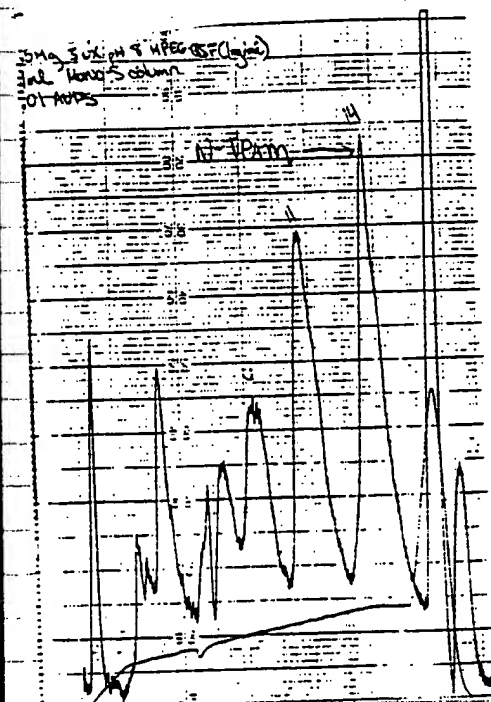
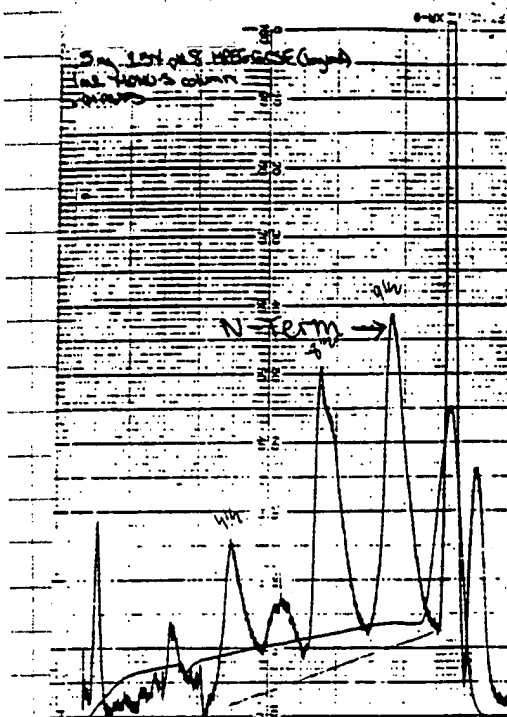
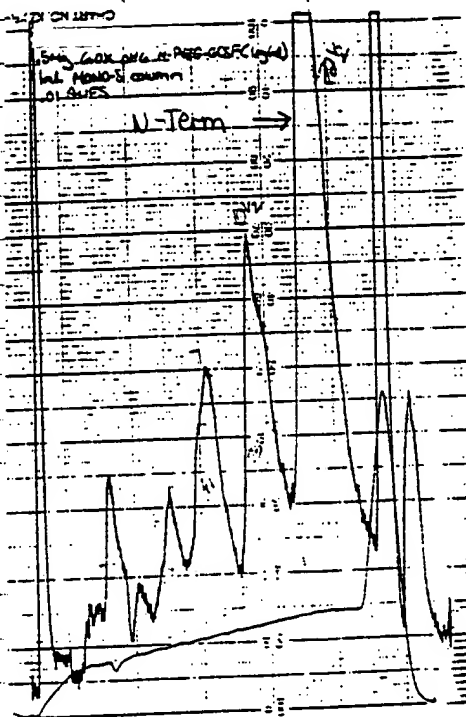
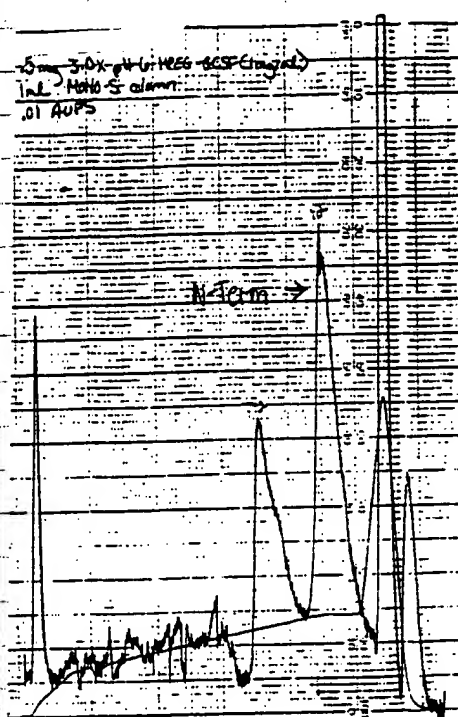
Christine Farnan

Date

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From Page No. 81

MONO-S IEX Profiles of Reaction @ Various pH's & Molar Ratios:



To Page No. 83

Witnessed & Understood by me,

Anne H. Gaa

Date

Invented by

Christine J. J. J.

Recorded by

Date

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From Page No. 82

Calculation of %'s by Integration of Peak Areas:

RECOVERY OF N-TERM PEG-GCSF

pH	Rxn	%PEG-GCSF	%MONO	%N-TERM OF MONO	%N-TERM OF TOTAL
5.75	15	72	72	60	31
6	6	60	75	56	25
8	3	68	64	45	20
6	3	36	87	62	19
8	1.5	40	82	42	14

$$\% \text{ PEG-GCSF} = 100 / (\% \text{ PEG} + \% \text{ MONO PEG}) \quad \text{p. 80}$$

$$\% \text{ MONO} = \% \text{ PEG-GCSF} / \% \text{ MONO PEG} \quad \text{p. 80}$$

$$\% \text{ N-TERM of MONO} = \% \text{ MONO PEG} / \% \text{ N-TERM PEG} \quad \text{p. 82}$$

$$\% \text{ N-TERM of Total} = (\% \text{ PEG-GCSF}) (\% \text{ MONO}) (\% \text{ N-TERM of MONO}) \quad \text{p. 83}$$

To Page No. X

Witnessed & Understood by me,

Amel H. Al-Hadi

Date

Invented by

Christine Jordan

Date

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Christine Turner

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D. Sherrin

Date

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C. Sherrin

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P. M. Kern

Date,

Invented by

Date

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C. F. Frazier

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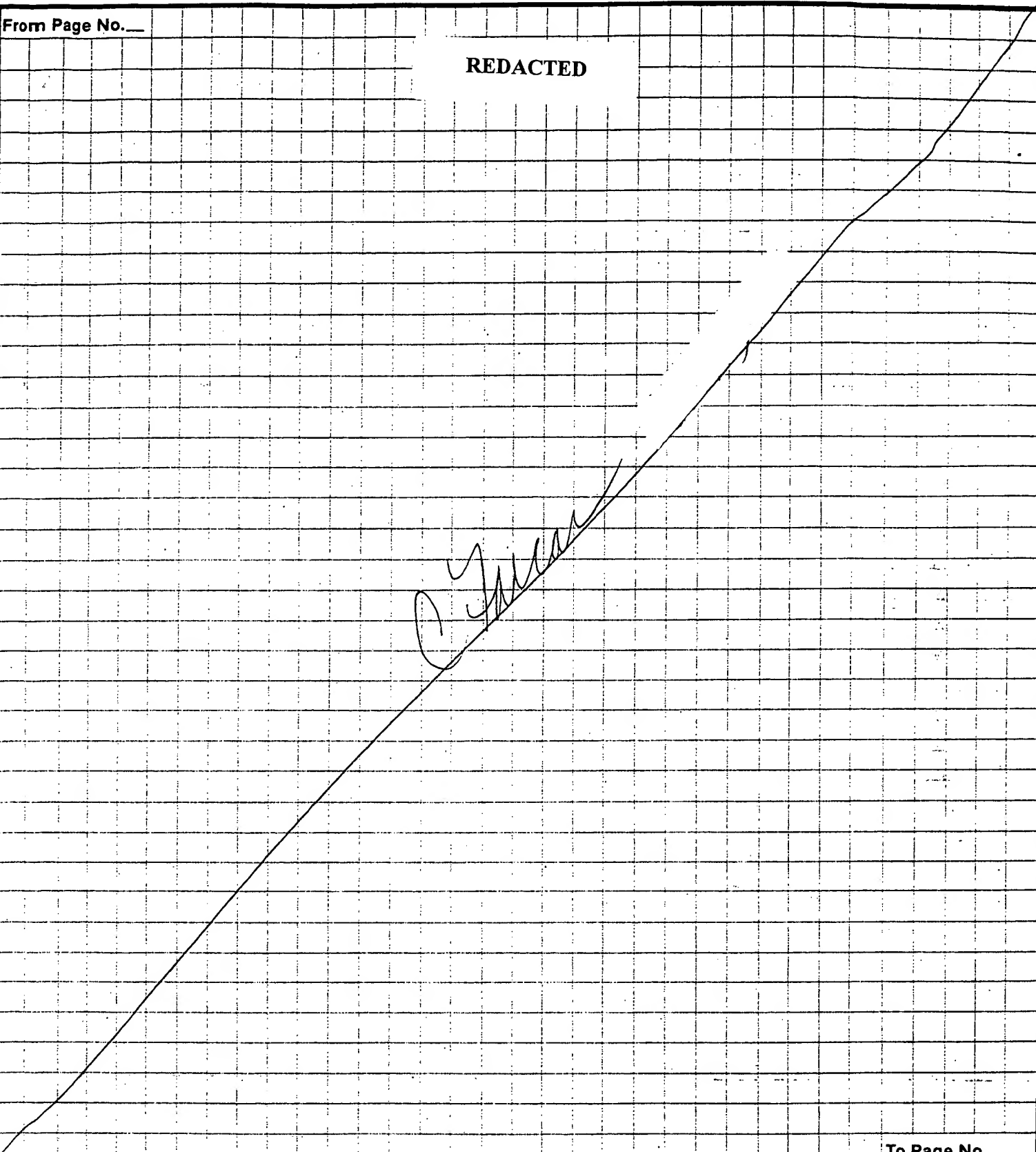
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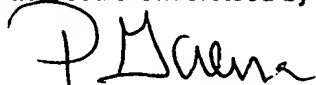
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